ENDOCRINOLOGY

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VOLUME 25

July–December, 1939

"*Just the place for a Snark! I have said it thrice:*
What I tell you three times is true."

. . . . . .

"*But oh, beamish nephew, beware of the day,*
If your Snark be a Boojum! For then
You will softly and suddenly vanish away,
And never be met with again!"

—LEWIS CARROLL. *The Hunting of the Snark.*

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Errata, Volume 24

Page 428, first abstract under 'Hypophysis.' For Marks, H. P., and F. G. Marks, *read* Marks, H. P., and F. G. Young.

Page 661, fourth line from bottom of page. Add: The thyroids of 30 controls averaged 17.6 mg.

Page 704, second paragraph under 'Methods.' For concentrated sulfuric acid *read* 5-normal sulfuric acid.

Page 816, fourteenth and fifteenth lines. For Bates, Riddle and Lahr (13) *read* Bates and Riddle (21).

Page 817, change ordinate numerals in figure 1 from 1, 2, and 3 *to* 0.5, 1.0 and 1.5

Page 818, ordinate in fig. 3, for per cent inhibitions *read* per cent inhibition.

Page 822, insert 21. Bates, R. W., and O. Riddle: *J. Pharmacol. & Exper. Therap.* 55: 365. 1935.

Page 901, sixth line of second column. For Marks, H. P., and F. G. Marks, *read* Marks, H. P., and F. G. Young.

ENDOCRINOLOGY

THE BULLETIN OF
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VOLUME 25 JULY, 1939 NUMBER 1

CERTAIN ACTIONS OF TESTOSTERONE ON THE ENDOMETRIUM OF THE MONKEY AND ON UTERINE BLEEDING[1,2]

EARL T. ENGLE AND PHILIP E. SMITH

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University

NEW YORK CITY

IT HAS BEEN reported that testosterone or testosterone propionate prevents the uterine bleeding of estrone withdrawal in macacus monkeys and inhibits the onset of menstruation in the normally cyclic monkey (1). In woman it is stated that the normal cycle is interrupted, with disappearance of the secretory (progestational) phase, often with regression and atrophy of the endometrium and postponement of, or but scanty menstruation (2).

Zuckerman (3) prevented the recurrence of bleeding in one normal monkey for 210 days during twice weekly injections of 25 mg. of testosterone propionate. A second animal was treated for 114 days, during which time no cyclic bleeding occurred. Bleeding did occur 8 days after the last injection. In the first animal at least, ovarian function was seriously disturbed. The endometrium illustrated by Zuckerman, while not atrophic, appears very inactive. Similar inactivation, or mild involution of the endometrium was reported by us after chronic estrogen treatment (4) and has been noted after chronic treatment with gonadotropic hormones. Thus, the morphological effect and perhaps also the suppression of cyclic bleeding is not a specific result of the testosterone treatment, but is a result achieved by the use of several endocrine agents. The inhibition of menstruation and production of atrophic endometria in women, as reported by Gaines et al. (2), may well be due to the action of testosterone on the hypophysis, as suggested by them and not to a direct effect on the endometrium.

Testosterone Propionate and Experimental Uterine Bleeding

The experiments here reported were performed on adult ovariectomized rhesus monkeys (*M. mulatta*). All animals were prepared by a standard

[1] Aided by a grant from the Rockefeller Foundation.

[2] The estrogen used in this study was progynon-B (Schering) and the progesterone was proluton (Schering), for both of which we are greatly indebted to Dr. Erwin Schwenk of the Schering Corporation. The testosterone used was perandren (Ciba), for which we are greatly indebted to Mr. Robert Mautner of the Ciba Corporation.

course of injections consisting of 400 I.U. estradiol in oil (progynon-B, Schering) daily for 10 days. Uterine bleeding uniformly follows cessation of this treatment, although it is prevented by treatment with progesterone.

Testosterone propionate (Ciba) in doses of 5 mg. per day for 15 days will prevent the expected bleeding after estrogen withdrawal. After cessation of testosterone treatment, bleeding occurred on days 8 and 10 (*My. 465:1*, *My. 470:1*). Bleeding occurred on day 12 following 25 mg. of testosterone propionate daily for 11 days (*My. 481:2*). In 2 others, a uterine biopsy was performed the day after the last injection of testosterone (*My. 470:2*, *My. 481:1*). In each of these 2 animals bleeding began on day 3 after cessation of testosterone injections.

In 2 other animals the estrogen series of treatments was followed by 10 days of treatment with progesterone (proluton, Schering), 1 mg. daily for 10 days (*My. 465:2*, *My. 481:3*).[3] Testosterone propionate in 5 mg. and 10 mg. daily doses, respectively, was then given, and bleeding occurred on day 4 and day 3 after cessation of progesterone, although they were receiving the testosterone injections.

In 3 additional animals, (*My. 479*, *My. 450*, *My. 497*) treated as before, the estrogen and progesterone (1 mg. daily for 10 days) treatments were followed by injections of 25 mg. of testosterone propionate per day for 10, 15 and 20 days. In these animals bleeding did not occur during the injections and the animals were killed.

Tissue Responses

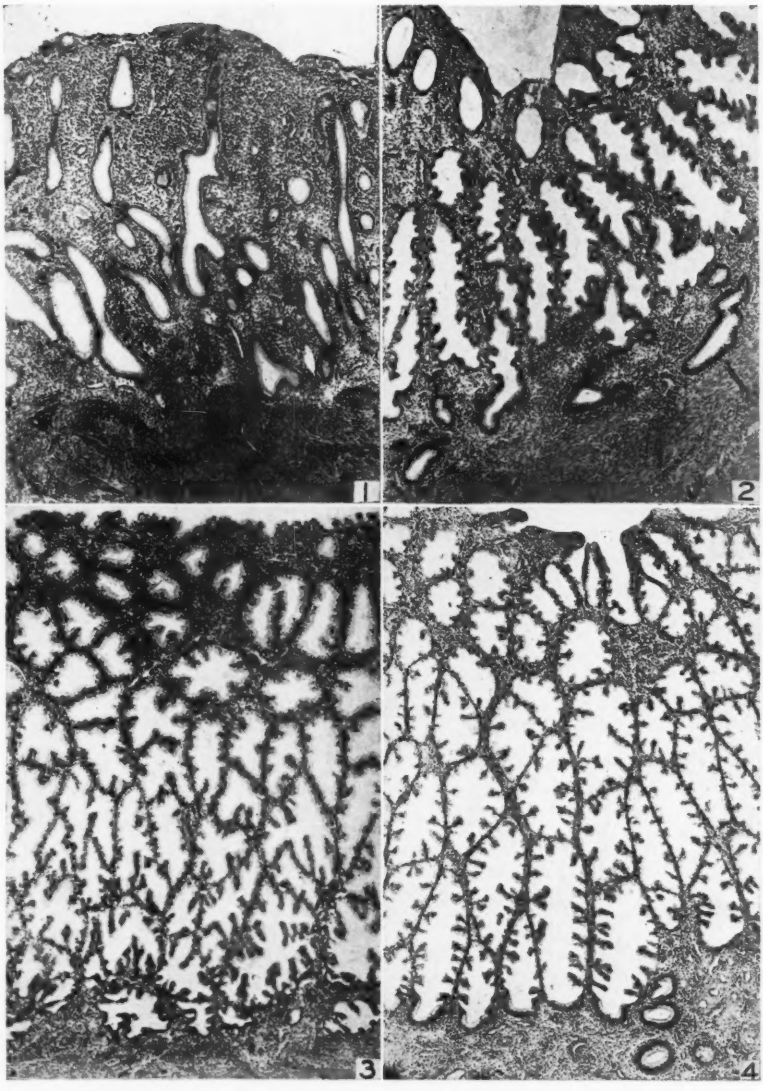
My. 470:2 received 10 days' treatment of estrogen followed by 5 mg. of testosterone propionate daily for 15 days. A specimen was obtained by biopsy on day 16. The character of the glands and stroma was essentially of the proliferative (estrogen) type (fig. 1). The glands were straight, the epithelium tall, columnar, with nuclei in the middle third of the cell. Abundant glycogen was present basally and apically. In many glands the epithelium is covered with a film of secretion, part of which was glycogen.

The endometrium of *My. 481:1* given the same treatment as *My. 470:2* above, cannot be histologically distinguished from a proliferative endometrium, except for an unusually dense compacta.

The endometria of animals treated with 25 mg. of testosterone daily, following progesterone, are quite unlike those pretreated with estrogen only. The glands have undergone pronounced secretory or progestational changes, considerably greater in degree than are induced in a monkey with 1 or 2 mg. of progesterone daily for 10 days. The glands have been thrown into highly folded structures with frond-like folds and projections of the mucosa. The cells are all highly secretory. The stroma is greatly reduced in amount. The lower portion of each gland in the basalis is alone unchanged, the epithelium being high columnar without secretory change.

A characteristic endometrium following testosterone therapy is that of *My. 450* (fig. 4, 8). This animal, following estrogen treatment for 10 days,

[3] Such treatment uniformly results in uterine bleeding on day 2 or 3 after cessation of progesterone injections.



ALL ANIMALS PRETREATED WITH ESTROGEN, (ESTRADIOL, PROGYNON-B, SCHERING). Fig. 1. My. 470:2 Biopsy specimen taken on day 16 of testosterone treatment. Fig. 2. Endometrium of My. 497. Testosterone following progesterone. Fig. 3. Endometrium of My. 471. Progesterone only for 30 days. Fig. 4. Endometrium of My. 450. Testosterone following progesterone.

had received progesterone (1 mg. daily) for 10 days and testosterone (25 mg. daily) for 15 days, a total of 25 days of progesterone-testosterone treatment. Another estrogen prepared animal, (My. 471) received a chronic treatment of progesterone only for 30 days, a total dose of 92 mg. being given. As

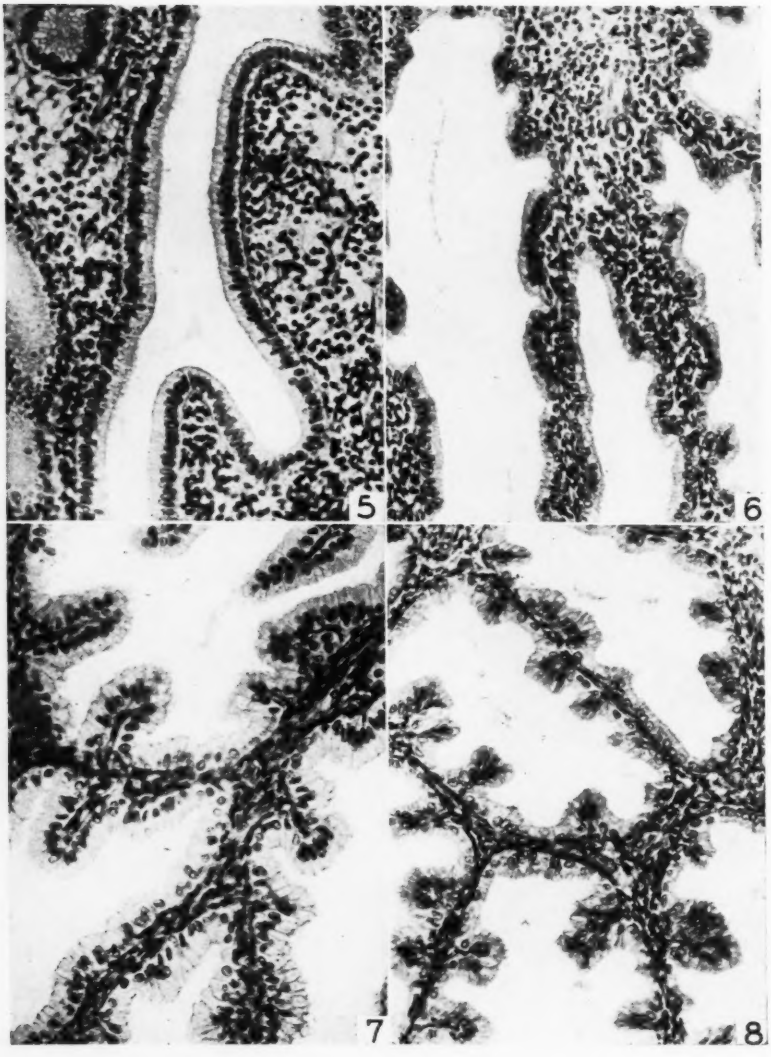


Fig. 5. My. 470:2. Higher magnification of section shown in fig. 1. Fig. 6. My. 497. Higher magnification of section shown in fig. 2. Fig. 7. My. 471. Higher magnification of section shown in fig. 3. Fig. 8. My. 450. Higher magnification of section shown in fig. 4.

is seen by comparing figures 3 and 7 with figures 4 and 8, there is a remarkable similarity between these two endometria. Glycogen, histologically determined, is abundant, and appears to be more abundant in the testosterone treated animals than in the progesterone series.

On a structural basis it appears that testosterone is able to maintain the

morphological characteristics of an estrogenically induced proliferative endometrium, without causing either involutional or secretory changes.

When progesterone is followed by testosterone in large doses, the structural characteristics of the progestational endometrium are maintained at least for 20 days by the testosterone.

Within the limits of time and dosage here reported, there is no indication that any involution of the endometrium has occurred. The only change of a pathological nature is the occurrence of isolated glandular cysts in specimens of both testosterone and progesterone animals. These are similar to those reported by Herrell as occurring in otherwise normal late secretory human endometria (5).

In all animals treated with testosterone, the sex skin response was uniform. In animals pretreated with estrogen, the color and edema remained

TABLE I

Monkey	Weight gm.	Estrogen	Progesterone	Testosterone	Uterine bleeding after last injection of testosterone
465:1	4780	400 I.U./d./10 days	none	5 mg./d./15 days	Bleeding, day 10
470:1	5000	Ibid.	none	Ibid.	Bleeding, day 8 (slight)
470:2	5000	Ibid.	none	Ibid.	Biopsy, day 16 of testosterone. Bleeding, day 3 and bled 7 days
481:1	5200	Ibid.	none	Ibid.	Biopsy, day 16 of testosterone. Bleeding, day 3 and bled 4 days
481:2	5200	Ibid.	none	25 mg./d./11 days	Bleeding, day 12
465:2	4780	Ibid.	1 mg./d./10 days	5 mg./d./15 days	Bleeding, day 4 after progesterone withdrawal
481:3	5200	Ibid.	Ibid.	10 mg./d./15 days	Bleeding, day 3 after progesterone withdrawal
479	6350	Ibid.	Ibid.	25 mg./d./10 days	Autopsy
450	3700	Ibid.	Ibid.	25 mg./d./15 days	Autopsy
497	4350	Ibid.	Ibid.	25 mg./d./20 days	Autopsy
471	4650	Ibid.	92 mg. in 30 days	none	Autopsy

unaltered during testosterone treatment. In animals treated with estrogen followed by progesterone, the color and turgescence faded rapidly during progesterone treatment. In the animals in which progesterone was followed by testosterone, the color and edema were restored to the estrogenic type induced by estrogen throughout the duration of testosterone treatment.

The results here reported may not be as greatly at variance with those published by Zuckerman and by Gaines et al. as would appear to be the case at first thought. In our experiments the proliferative and the secretory states of the endometrium had been established before the testosterone treatments were begun. The testosterone action has then been superimposed on this existing endometrial condition. In the work from the other two laboratories it seems probable, although the stage of the cycle when the testosterone treatment was begun is not stated in the papers of Gaines et al., that the treatments extended throughout the cycle and were begun before even the proliferative stage had developed. The treatments, by repressing the hypophysis and thereby indirectly preventing secretion by the ovary,

would prevent the development of the proliferative or the secretory stages upon which the testosterone would act.

CONCLUSIONS

From the foregoing material the following observations may be made.

Uterine bleeding of estrogen privea may be prevented by 5 mg. of testosterone propionate daily.

The interval between cessation of testosterone propionate treatment in estrogen pretreated monkeys and the appearance of bleeding is similar to that of estrogen withdrawal.

The endometrium of such estrogen-testosterone treated monkeys is essentially a proliferative type, with perhaps more glycogen than is usual in an estrogen prepared endometrium.

The sex skin response is typically estrin-like.

When estrogen is followed by progesterone, uterine bleeding was not prevented by either 5 mg. or 10 mg. of testosterone propionate, but was postponed by 25 mg. daily for 10, 15 and 20 days, respectively.

The endometria of these animals receiving total doses of 250 mg., 375 mg. and 500 mg. of testosterone propionate after progesterone are essentially progestational in character.

No indication of atrophy of the endometrium has been observed, in animals previously prepared with estrogen.

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INFLUENCE OF ULTRAVIOLET IRRADIATION UPON EXCRETION OF SEX HORMONES IN THE MALE[1]

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A WEALTH OF STUDIES on the influence of light upon the sexual cycle, pituitary activity, fertility, etc., have been carried out in recent years (1). Among the external factors which are susceptible of influencing sexual activity, light varying according to seasons and climates is included by Benoit (2) as of first importance. Observations by Bissonnette and Csech (3) that hens with additional light during the night start egg laying several months earlier than breeds of the same age but without additional light, have been confirmed and are now completely accepted.

Bissonnette, as well as Benoit, stresses the opinion that stimulation of the pituitary is the primary effect of increased light, and that the pituitary in turn increases the activity of the gonads. Hoskins and Rioch (1), discussing Bissonnette's paper, object to this idea, believing that there are different ways of explaining the experimental results.

The work of Witschi (4), and of Riley and Witschi (5), is important in several respects. These authors conclude from their experiments that there is only a low degree of responsiveness of the female pituitary to light if a 100-watt incandescent bulb is applied. Light applied for long periods gave no greater results than when applied for short periods. One result of the investigation of these authors may be stressed, namely, that "it appears that the ovary of the bird is harder to stimulate than the testis." Moreover, Witschi (4), in his earlier work, mentions several of his own observations in different groups of birds which indicate that "if light has any influence, then this group of birds reacts to shortening and not to lengthening the day." Marshall (6) reports similar observations in birds as well as in sheep and deer. Thus, although there is little or no doubt that light exerts an influence upon the sexual cycle, there still is doubt as to the manner in which its influence in different species is exerted, and whether increasing or decreasing light is of importance.

It must be stated that there is no agreement concerning the question whether irradiation of the skin is important in exerting an influence upon the sexual cycle or not. Parkes (7), in a different connection, states that the peculiarity of the Seabright Bantam lies in the skin which is highly sensitive to estrogens. Bissonnette (1), on the other hand, states that irradiation of the bare skin is negligible in its effect in comparison with the mode of entrance of the light rays through the eyes (photo-pituitary reaction).

More closely related to our own experiments is the controversy whether ultraviolet irradiation is more potent than other forms of irradiation. While Bissonnette found that ultraviolet irradiation is not effective or necessary in starlings, Marshall

[1] This investigation was aided by grants from the Commonwealth of Massachusetts, the Rockefeller Foundation, and the Emergency Committee in Aid of Displaced Foreign Medical Scientists.

(6), on the contrary, found that ultraviolet irradiation was much more effective than luminous irradiation; that ultraviolet irradiation caused a prolonged result after the irradiation had ceased. "The presumption is that ultraviolet irradiation has a semi-permanent influence on the hypophysis which is thereby made to undergo a prolonged period of hyperpituitarism in regard to the production of the gonadotropic hormone." Baker (according to Marshall) suggested that a similar influence may account for the two breeding seasons of many tropical birds, "these seasons occurring at times when the ultraviolet radiation from the sun is probably at its greatest." Rowan (8) in his recent paper states that a standard electric light gives the same results in sparrows as an ultraviolet sun-lamp.

Applying these physiological results in animals to man, we are thus faced with the following questions.

a), Does ultraviolet irradiation exert an influence upon the formation and excretion of sex hormones?

b), If so, is the irradiation of the skin the most important single factor, or is the photo-pituitary reaction of greater importance?

c), The third question with which this paper deals is, what influence is exerted by ultraviolet irradiation of presumably indifferent parts of the skin as compared with irradiation of the genital region?

PROCEDURE

A small, quartz, mercury lamp, now on the market, was used. According to the information from the factory, the spectrum of this lamp consists of 52% infra-red, 20% luminous and 28% ultraviolet rays. The duration of exposure to the light was 8, 10, 12, 15, 18 and 20 minutes, each succeeding exposure being increased over the preceding one. There was in the majority of cases a marked erythema but no undesirable skin reaction and the procedure of increased exposure could be applied in all cases.

We started with a group of patients, improvement of whose general condition by ultraviolet irradiation we believed would have a beneficial influence upon their mental state as well as bearing upon the experimental problem. All patients suffered from depressive states; 3 patients were 54 years of age, representing depressive phases of manic-depressive psychosis; 2 patients were 28 and 45 years of age respectively, psychopathias with depressive features. The history of the cases may be omitted.

As shown below, successively different parts of the body were irradiated, namely, the anterior part of the body including chest, face and genitalia; chest and head alone; genital region alone; total back; and a small portion of the back in the region of the scapulae corresponding in size to the area of the genitalia. The non-exposed parts of the body were covered either by the garments and underwear of the patients or by dark clothes.

For the determination of sex hormone excretion, we followed the method recently published by one of us (9). A similar colorimetric method was published a few months ago in England by Callow (10). The advantages of colorimetric methods over biological assays for serial examinations are manifold. The exactness of the colorimetric assay is as great as that of the biological methods and probably in the near future will be greater.

We have collected some data on 'female' hormones, but this report deals only with the formation and excretion of androsterones in urine. Since nothing is known at the present time about the storage of male sex hormones in the body, their greater excretion may be regarded as an indication of their greater formation in the body, although this is not certain.

EXPERIMENTAL RESULTS

The results in our group of patients up to the present have been surprisingly uniform. Figure 1 demonstrates the typical reaction. The androsterone excretion, determined twice before starting irradiation, was about 70 I.U. androsterone per liter (and approximately per day). After 5 irradiations of the chest, the hormone output was raised to 155 I.U. per liter, that is, an increase of 120%. These 5 irradiations were given in 5 consecutive days in the usual manner of application of ultraviolet light for medical purposes, namely, the eyes are closed and, in addition, covered with dark glasses. In this particular case, head, chest, genitalia and thighs were exposed to irradiation, the light being focussed on the lower thorax and upper abdomen. After an interval of 8 days without irradiation, the hormone output

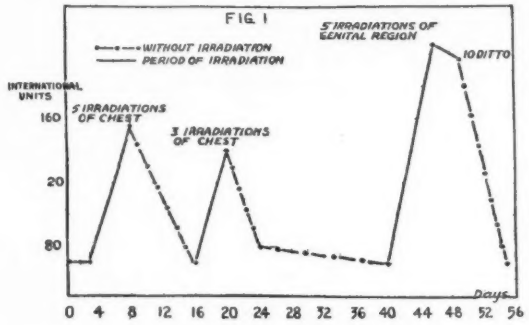


Fig. 1. CHART SHOWING THAT THE ANDROSTERONE OUTPUT INCREASES IN ACCORDANCE WITH THE NUMBER OF IRRADIATIONS AND FURTHERMORE THAT IRRADIATION OF THE GENITAL REGION IS MORE EFFECTIVE THAN IRRADIATION OF THE CHEST.

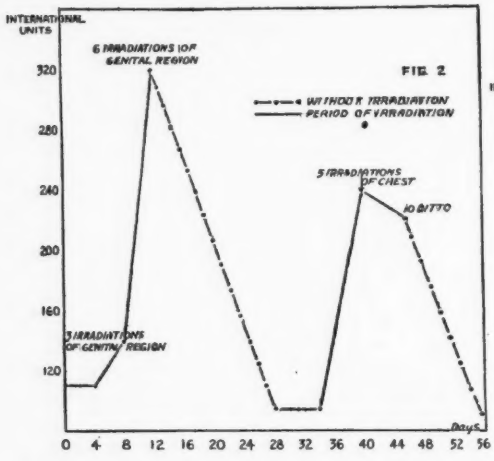
went back to its previous level of about 70 I.U. androsterone per liter. Three more irradiations, given over a period of 5 days, raised the hormone level from 70 to 140 I.U. per liter (fig. 1). After an interval of 5 days without treatment, the output fell back to 80 I.U. per liter, and another examination 2½ weeks later showed it to be again 70 I.U. per liter.

The next series of irradiation was applied solely to the genital region and its immediate vicinity, the remainder of the body being carefully protected from all rays. After 5 irradiations during 6 days, the androsterone excretion was raised from 70 to 205 I.U., nearly 200%. Five more irradiations during the next 5 days did not further raise the hormone output, but it persisted at nearly the same level. After another 10-day interval without irradiation, the hormone output went back to its 'normal' level of about 70 I.U. per liter (fig. 1).

The findings in this case merit the emphasis of repetition, namely, that 5 irradiations are more effective than 3 irradiations; furthermore, that 10 irradiations are not more effective than 5 irradiations. We must assume that the peak of the reaction is reached either by 5 irradiations or at about that number. Finally, it is to be stressed that irradiation of the genital region is more effective than irradiation of other, even much larger, parts of the body.

In all cases in which irradiation was applied on consecutive or nearly consecutive days, a peak was found after about 5 irradiations. A case in which irradiations were applied twice weekly at first and later only once weekly did not show the peak after 5 irradiations, but rather a continuous rise during the whole period of treatment which reached the number of 12 irradiations.

That the greater response after irradiation of the genital region is not caused by a previous sensitization of the pituitary is shown by figure 2, in which the series of irradiations of the genital region was first applied, and in a second series head and thorax were exposed and the genital region covered. As on the previous occasions, irradiation of the genital region proved to be more effective (a rise from 110 to 320 I.U. per liter after 6 irradiations), than irradiation of even much larger, non-genital parts of the body.



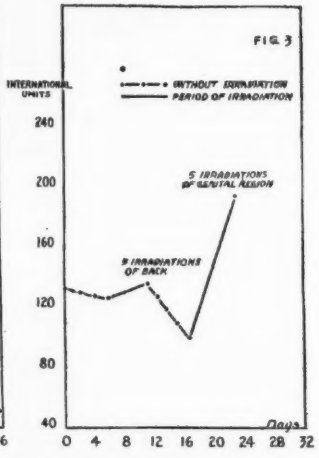


Fig. 2. Chart showing that irradiation of the genital region is more effective than irradiation of the chest, if the possibility of previous sensitization is excluded. Fig. 3. Chart showing that irradiation of a small area of skin, corresponding in size to the genital region, is not effective, while irradiation of the genital region definitely raises androsterone execretion.

Although irradiation of the head, thorax and abdomen gave an appreciable rise (from 90 to 240 I.U.), it was impossible to raise the level of hormone output by further irradiation of the chest to the height reached by irradiation of the genital region (fig. 2).

Irradiation of various non-genital parts of the body gave identical results if the size of the irradiated parts was identical, for example, a rise from 70 to 150 I.U. per liter after irradiation of the front of the body from the forehead to the umbilicus or a rise from 90 to 180 I.U. per liter after irradiation of the neck and back.

In order to corroborate the observation made from the above mentioned experiments, namely, that irradiation of the genitals and their vicinity is more important than irradiation of other parts of the skin, the following procedure was carried out in 2 patients. First, the patients received 5 irradiations of the region of the scapulae over an area of about 20×50 cm., the

rest of the body being covered. After an interval of 8 days without treatment, the same patients received 5 irradiations of the genital region, about the same square area being exposed and the rest of the body completely covered. Figure 3 shows this result clearly, namely, that after irradiation of a small area of the back, there is no rise of the hormone excretion in the urine, while irradiation of an area of the same size in the genital region shows a definite rise of the androsterone excretion.

It may be added that the rise in female hormones is slower, lower and more retarded than that of male hormones; but our observations concerning female hormones are only casual and incomplete.

DISCUSSION

In discussing our experiments, we may first state that any relation of the depressive mental states of our patients to the formation and excretion of male hormones is not discernible. It may be said, however, that the ultraviolet irradiation and/or the increase of hormones exercised a good influence on the 2 patients who were not true manic-depressives, while the mental state of the other 3 patients remained unaffected.

A question which might be discussed first is whether the observed changes are of any significance at all or whether they are rather physiological variations. Gallagher and his coworkers (11) found in one individual a range of variation from 13 to 69 I.U. androsterone per day, and similar variations in other individuals. From findings of this kind, one might conclude that variations of the degree of our findings were insignificant, but we cannot confirm the findings of Gallagher et al. On repeated urine examinations of the same individual, either on consecutive days or after weeks or months, we obtained results of a highly constant uniformity. Gallagher's and our findings are not necessarily contradictory, although they seem to be. If hormone excretion is partly dependent on external influences, then it is not surprising that there is a range of variation in the free-living individual. On the other hand, we are here dealing with hospitalized patients, all of whom live for months under constant conditions. The uniformity of their living conditions may explain the uniformity of our findings in repeated urine examinations. Therefore, we can regard our patients as living under fairly good experimental conditions, the only change or new variable being the application of ultraviolet irradiation. From the sum total of our experience, we may safely state that under the circumstances in which our patients live, the uniform change in the excretion of androsterones is definitely significant and not coincident.

Another point may be stressed, that is, a possible seasonal factor which might influence our results. Our experiments were carried on from November 20, 1938 to February 10, 1939, a season with a low degree of sunshine. It is a problem for later investigation whether the experiments can be repeated with the same result in the spring or summer.

It is not the purpose of this paper to discuss the value of colorimetric hormone assays in detail. Callow, Callow and Emmens (12) state "that the correlation between biological and colorimetric assays is not much less than that between biological assay and the true amount of androgenic material

present" and later "there remains no doubt, however, that the greater part of the departure of the correlation between colorimetric and biological assays from unity is due to the errors of biological assay." These citations check with our own experience. We found that the main chromogens in urine which might influence the reaction with Zimmermann's (13) reagent are removed by the kind of extraction applied. We do not wish to discuss in more detail at this point the value of the colorimetric measurement of androsterone-excretion but only to stress that our experience with this kind of assay is both favorable and reliable. However, a series of biological assays will be carried out as soon as a new series of patients is started.

The next question which merits discussion is whether it is the ultraviolet part of the light band which causes the increase in excretion of androgens. To speak of ultraviolet irradiation in the everyday practice of medicine is to mean the application of the mercury quartz lamp. There are many hints that it is really the ultraviolet part of the spectrum which is of the greatest importance in bringing about the result observed. But a further investigation by the use of narrow and selected spectral bands is planned.

There is plenty of room for speculation as to whether and how the cholesterol and possibly other sterols of the skin are mobilized by irradiation and transformed to sex sterols. But this is mere speculation and our experiments disclose no mechanism of production of sex hormones except that the genital zone is particularly important. The thin and transparent scrotum may not absorb the ultraviolet rays entirely and the testes may be directly stimulated to higher activity. There is no evidence, however, that the pituitary plays an important rôle in the stimulation of increased androsterone formation and output in our experiments.

SUMMARY

Ultraviolet irradiation applied with a mercury quartz lamp increases the excretion of androsterone in urine. Measurements were made colorimetrically. Irradiation of the scrotum and its vicinity proves to be more effective than irradiation of other parts of the skin. Our experiments furnish no proof that the pituitary plays a primary rôle in the stimulation of increased androgenic activity.

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SOME CLINICAL OBSERVATIONS UPON THE METABOLISM AND UTILIZATION OF CRYSTALLINE PROGESTERONE

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IN TWO RECENT communications (1, 2) by members of our group, attention was directed to a failure to observe any significant alterations in the urinary titers of sodium pregnandiol glucuronide of patients with various functional irregularities of uterine bleeding following intramuscular injections of oily solutions of crystalline progesterone. These data and those also reported regarding the infrequent occurrence of specific endometriotropic responses suggested that, perhaps, ineffective metabolism of crystalline progesterone was related to its inadequate utilization by the endometrium. Stover and Pratt (3) have reported recently that they also had failed to recover any of the pregnandiol complex from the urine of patients who had been given intramuscular injections of an active extract of corpora lutea. Venning and Browne (4), however, had described the recovery of amounts of the pregnandiol complex comparable to 46 to 12% of that expected from the injected progesterone. The purpose of the present communication is to present additional data regarding the metabolism and utilization of crystalline progesterone.

METHOD OF STUDY

Two groups of patients were studied, one primarily in reference to endometriotropic responses, the other chiefly regarding the urinary excretion of sodium pregnandiol glucuronide. All of these had various degrees of alteration of ovarian function and all presented irregularities of bleeding as common symptomatology. All received some modification of a therapeutic regime, employing cyclic administration of estrogens and/or progesterone, as previously described by one of us (1). Endometriotropic responses were studied from endometrial biopsies obtained at the onset of flowing or within the first 18 hours of its beginning. Microscopic study of all endometrial tissue was made by one of us (E.C.H.) and the endometrial patterns were classified by a system previously described (5). Daily 24-hour specimens of urines were studied during the non-bleeding phases of the patients in the second group for titers of sodium pregnandiol glucuronide. The method described by Venning (6) was employed.

Analysis of Endometriotropic Data

These data were secured during 117 series of therapy given 23 patients. The cyclic treatments[1] were distributed for the most part as follows.

Estrogens given in daily doses of 10,000 I.U. for 12 to 14 days followed with subsequent daily injections of 5 mg. of progesterone for 2 to 10 days—46 cycles.

Estrogens given similarly, followed by daily injections of 10,000 I.U. of estrogens and 5 mg. of progesterone for 7 to 10 days—21 cycles.

Estrogens and progesterone, in daily doses of 10,000 I.U. and 5 mg., respectively, given for 7 to 10 days—9 cycles.

Progesterone in daily doses of 5 mg. for 7 to 10 days—41 cycles.

The total amount of progesterone given was 4045 mg. The average total dose for a cycle of therapy was 34.5 mg.

The endometriotropic responses were sampled by biopsies in 99 of the 117 cycles. Some degree of progestational alteration was encountered in 22 instances: 21 specimens were classified as mixed endometria, 1 as normal menstrual. Five of these progestational responses occurred during the 9 cycles of therapy during which estrogens and progesterone were given concurrently. Only 8 of these 9 cycles were investigated by biopsy.

Analysis of Data on Excretion of Pregnandiol Complex

These data were secured during 40 cycles of 7 patients. Cyclic therapy similar in kind and dosage to that described in the previous section was given during 23 cycles. Six of the 7 patients excreted varying amounts of the pregnandiol complex prior to therapy, despite endometriotropic evidence of ineffective endometrial utilization. The following arbitrary rules were employed in collecting the data to be presented: no determinations were done during episodes of bleeding; no therapy was included if urines were not available for at least 24 hours afterwards; all of the pregnandiol complex excreted for 72 hours after the last injection of progesterone was credited to therapy.

The following data were obtained.

During the administration of progesterone alone: 435 mg. were given and 95 urines extracted for the pregnandiol complex with the recovery of a total of 61 mg., which represented an average recovery per determination of 0.64 mg. and a total theoretical[2] yield of 8.2%.

During the administration concurrently of estrogens and progesterone: 140 mg. of progesterone and 270,000 I.U. of estrogens were given and 29 urines extracted for the pregnandiol complex with the recovery of 37 mg., which represented an average recovery per determination of 1.27 mg. and a total theoretical yield of 15.5%.

During the administration of estrogens alone: 1,990,000 I.U. were given and 216 urines extracted for the pregnandiol complex with the recovery of 285.4 mg. which represented an average recovery per determination of 1.32 mg.

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[1] The estrogens employed were estrone (theelin, Parke, Davis & Co., and amniotin, E. R. Squibb & Sons) and estradiol benzoate (progynon-B, Schering Corp.) Progesterone (proluton, Schering Corp.) was used.

[2] This was calculated upon the assumption that each molecule of progesterone (molecular weight 314) is converted into a molecule of sodium pregnandiol glucuronide (molecular weight 536); thus, the amount of progesterone in mg. x536/314 or 1.7 = theoretical yield of sodium pregnandiol glucuronide in mg.

During intervals of no therapy: 186 urines were extracted for the pregnandiol complex with the recovery of 340 mg., which represented an average recovery per determination of 1.84 mg.

DISCUSSION

The efficiency of the therapy given these patients, when judged on the basis of the observed endometriotropic responses was 22% if any degree of progestational alteration is accepted as an end-point, but only 1% if the production of a normal progestational endometrium is held to be a criterion of specific action.

Interpretation of the data regarding the urinary values of the pregnandiol complex must take into consideration that 6 of the 7 patients exhibited spontaneous metabolisms of their intrinsic progestin. The average recovery per determination of the pregnandiol complex during intervals when no therapy was given was 1.84 mg. Even, when one unusual cycle of one of these patients, during which 29 determinations were done and 118 mg. of the pregnandiol complex was recovered, is omitted from consideration, the average value for a determination is 1.41 mg. It seems warranted, therefore, to assume from these data that the average recovery per determination of 0.64 mg. of the pregnandiol complex obtained during the administration of progesterone alone represents not only a failure of metabolism of the progesterone injected, but also a depression of the spontaneous metabolism of intrinsic progestin. It is worthy of note that the administration of 340 of the 435 mg. of progesterone given alone was followed by no excretion of the pregnandiol complex.

Since the average recoveries per determination during the administration concurrently of estrogens and progesterone, during that of estrogens alone and during intervals of no therapy are so nearly equal, it seems to be a likely assumption that when estrogens are given along with progesterone, they prevent depression of the spontaneous metabolism of progestin. No evidence can be deduced from these data upon the urinary titers of the pregnandiol complex that estrogens enhance the metabolism of injected progesterone. The endometriotropic data suggest such an influence.

The inferences permitted from these data should be limited strictly to the conditions which existed during this investigation. The endocrine status of the patients treated, the dosage and method of administration of the sterols (estrone, estradiol-benzoate, and progesterone) the technic of endometrial biopsy, and the method employed for urinary estimations of the pregnandiol complex must be considered in evaluating these results. Similar studies on oöphorectomized women should be done, as these would not be complicated by the existence of a metabolism of endogenous progestin.

The ultimate fate of the progesterone injected, but apparently not metabolized, has not been determined. At present some of our group are carrying out biossays for progesterone upon the urines of these patients to ascertain if this sterol is excreted unaltered.

SUMMARY

Endometriotropic responses were studied during 99 of 117 cycles of therapy with estrogens and/or progesterone given 23 women. Some degree of

progestational alteration was encountered at the end of 22 cycles, but only one normal progestational endometrium occurred, the remainder being classified as mixed. Urinary titers of sodium pregnandiol glucuronide were investigated during 40 cycles of 7 women, who received similar therapy during 23 of these cycles. No evidence was encountered in these studies that crystalline progesterone, when given intramuscularly in oily solution is metabolized into sodium pregnandiol glucuronide. Apparently, when given alone, progesterone depresses the spontaneous metabolism of endogenous progestin. Endometriotropic studies suggest the possibility that the concurrent administration of estrogens with progesterone may enhance the utilization of the latter. The ineffective utilization of crystalline progesterone by the endometria of these patients seems related to an inadequate metabolism of that sterol.

We are indebted to Schering Corp., Bloomfield, N. J., to Parke, Davis & Co., Detroit, Mich., and to E. R. Squibb & Sons, New York City, for liberal supplies of the sterols used in these studies. A part of the expenses incurred in this investigation was defrayed by a grant of the Research Council of Duke University to one of us (E.C.H.).

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IMMUNOLOGIC INVESTIGATION OF HYPOPHYSEAL MAMMOTROPIC PREPARATIONS

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THE ONLY EXPERIMENTS dealing with antisubstances to the lactogenic or mammotropic hormone that we are aware of are those of Kabak and Stulova (1) who induced a refractory state to prolactin in chronically injected pigeons, and Young (2) and Strangeways (3) who produced antisera against preparations of prolactin that still contained blood proteins.

In our investigation we were interested in determining *a*), whether our mammotropic preparations were antigenic, *b*), whether they were species-specific antigens, *c*), whether the antibodies formed against them were capable of inhibiting their physiological activity, and *d*), whether their antihormonal properties were species-specific.

METHODS AND RESULTS

The beef and sheep mammotropic preparations used were made according to methods previously published (4). They stimulated pigeon crop growth in a total dose of 0.1 mg. when given subcutaneously for 4 days (macro-test), and caused milk secretion in post-estrous virgin guinea pigs in a total dose of 1.0 mg. also injected subcutaneously for 4 days. Previous studies have convinced us that these preparations contain little if any of the other easily recognizable pituitary hormones, and serologic tests have shown that serum proteins are absent from them. Chemical studies have shown that mammotropin contains 15 to 16% protein nitrogen, and it gives positive biuret, ninhydrin, Millon, Hopkins-Cole and xanthoproteic tests. All of the preparations used in this study had been precipitated isoelectrically at pH 5.5 and gave non-pigmented, water-clear solutions (2 to 4%) when dissolved in water with the aid of a small amount of weak NaOH, and adjusted to pH 7.4 with HCl.

Experiments with anaphylaxis. Antigenicity of mammotropin was readily established by the anaphylactic test in young male guinea pigs. Some animals were sensitized by intraperitoneal injections of small amounts of antigen, and tested 2 to 3 weeks later by intracardial injections of larger doses, while others were given large sensitizing doses intracardially in order to control the possibility of primary shock from protein-split products. In no instance was there the slightest suggestion of shock upon primary injection of over 20 guinea pigs with doses as high as 20 mg., whereas as little as 12γ sufficed to kill sensitized animals. In order to answer a possible objection that serum proteins might be present in our extracts, acting as antigens, guinea pigs

[1] Aided by a grant from the Board of Research of the University of California.

TABLE 1. ANAPHYLACTIC REACTIONS IN GUINEA PIGS SENSITIZED TO BEEF OR SHEEP MAMMOTROPIN

Group	Guinea Pig	Sensitized to	Shocked with	Degree of Shock
1	8, 9, 10	1.5 mg. 209S[1]	10 mg. 211S	Lethal
	11, 13, 14	1.5 mg. 209S	10 mg. 205B	Lethal
2	1, 2, 3	2.7 mg. 205B[1]	10 mg. 205B	Lethal
	4, 6	2.7 mg. 205B	10 mg. 211S	Lethal
	5	2.7 mg. 205B	10 mg. 211S	Severe
3	53	20 mg. 228B	0.012 mg. 230S	Lethal
	55	20 mg. 228B	0.012 mg. 228B	Moderate
4	48	20 mg. 230S	10 mg. 230S	Lethal
	50	20 mg. 230S	6 mg. 228B	Lethal
	52	20 mg. 230S	1.2 mg. 228B	Lethal
	64	20 mg. 230S	0.12 mg. 228B	Lethal
	63	20 mg. 230S	0.012 mg. 228B	Moderate
	62	20 mg. 230S	0.012 mg. 230S	Moderate

[1] Throughout this paper S indicates that the mammotropin was made from sheep pituitaries; B, from beef pituitaries.

sensitized to beef or sheep mammotropin were injected with beef and sheep sera and showed no anaphylaxis.

The results of the anaphylaxis experiments are summarized in table 1. In group 1 death followed intracardial injection of 10 mg. of either beef or sheep mammotropin in animals sensitized by intraperitoneal injection of 1.5 mg. of sheep mammotropin (SM). In group 2, the same results were obtained in animals sensitized to 2.7 mg. of beef mammotropin (BM) (animal 5, injected with heterologous antigen, showed severe symptoms and recovered). In group 3, animal 53, sensitized with 20 mg. BM, was fatally shocked with 0.012 mg. of SM, while animal 55, similarly sensitized, showed partial shock with recovery in response to 0.012 mg. BM. In group 4, guinea pigs 62 and 63 after sensitization to 20 mg. SM received 0.012 mg. of SM and BM respectively, and both showed equally moderate shock symptoms with recovery. Animals 48, 50, 52, 64, and 13 others not included in the table were used to determine critical sensitizing and shocking doses.

Experiments with passive sensitization. The presence of anaphylactins in the blood of guinea pigs sensitized by one injection of BM or SM was shown by passive sensitization. Animals 65 and 66 (table 2) were injected intraperitoneally with 2.5 cc. of serum from guinea pigs actively sensitized 3 weeks previously to BM. After 24 hours, the former was fatally shocked with 1 mg. BM, and the latter with 1 mg. SM given intracardially. Guinea pigs

TABLE 2. ANAPHYLACTIC REACTIONS IN GUINEA PIGS PASSIVELY SENSITIZED WITH 2.5 CC. OF SERA OF GUINEA PIGS ACTIVELY SENSITIZED TO BEEF AND SHEEP MAMMOTROPIN

Donor	Sensitized to	Recipient	Shocked with	Degree of Shock
53	228B	65	1.0 mg. 228B	Lethal
55	228B	66	1.0 mg. 230S	Lethal
62	230S	67	1.0 mg. 228B	Lethal
63	230S	69	1.0 mg. 230S	Lethal
64	230S	68	0.1 mg. 228B	Slight

67, 68, 69, were passively sensitized to SM, and after 24 hours, animal 67 was shocked with 1 mg. BM, and animal 69 with 1 mg. SM. Animal 68 suffered mild shock when injected with 0.1 mg. BM. A nonsensitized animal showed no reaction to 1 mg. doses of BM and SM.

It was concluded from the anaphylaxis experiments that our mammotropic preparations were antigenic and possibly species-non-specific.

Experiments with the Dale reaction. The Dale uterine strip reaction offers a much better means of judging antigenic specificity, since one may test different antigens in varying dilutions on a single hypersensitive animal. Young, normal, virgin guinea pigs were sensitized by intraperitoneal injections of 20 mg. BM or SM. One month later they were killed, and each uterine horn sectioned to give proximal and distal halves. One of the strips was placed immediately in Locke-Ringer solution at 38° C., and stabilized on a muscle lever for a kymographic record. The others were stored in Locke-Ringer solution in the icebox for 1 to 2 hours until needed. When a strip had become stabilized as shown by its rhythmic contractions, various test solutions were added to the bath and the reactions recorded (table 3). Strip 2 of animal 54 (fig. 1) sensitized to 20 mg. BM showed no reaction to beef serum, but reacted strongly to 1 mg. BM (228), and was then found desensitized to higher doses of both BM and SM (230). Strip 1 reacted equally well to 1 mg. SM and a slight residual sensitivity was then found to 12 mg. BM. Animal 56, sensitized to BM was used to show the minimal amount of mammotropin to which the uterus would respond, as well as to show the desensitizing dose. No reaction occurred in strip 2 until the addition of 0.1 mg. of BM (fig. 2). Residual sensitivity was demonstrated by a second reaction—this time to 1.0 mg.—but no further reaction took place when 10 mg. of BM and SM were added. Strip 1 exposed to increasing doses of SM showed precisely the same reactions as strip 2. Strips 1 and 2 of guinea pig 58 reacted completely to 10 mg. of BM and SM respectively, and then showed no residual sensitivity to the sensitizing antigen (fig. 3).

In order to determine whether the chemical treatment of beef and sheep mammotropin had denatured or rendered identical two antigenically different proteins, tests were done on guinea pig 59 with saline suspensions of finely ground, freshly dissected anterior lobes. These suspensions were found to have no oxytocic effect on normal guinea pig uteri in the doses used, but did cause typical contraction in guinea pigs sensitized to either BM or SM. Strip 1 of guinea pig 59 showed a strong reaction to 500 mg. of sheep anterior lobe suspension and was subsequently found desensitized when tested with 200 mg. of the same suspension, then 10 and 45 mg. of the SM used in sensitizing the animal. Strip 2 reacted likewise to a beef saline suspension, and was found insensitive to 10 mg. of SM. Conversely, 10 mg. of SM caused a strong contraction in strip 3 and the strip was then found insensitive to a beef saline suspension. It seemed unlikely, therefore, that the chemical treatment had altered the preparations antigenically.

Strip 1 of guinea pig 60 sensitized to SM showed no reaction to sheep serum, and then responded strongly to 10 mg. SM which desensitized it.

TABLE 3. DALE REACTIONS TO BEEF AND SHEEP MAMMOTROPIN

Guinea pig	Sensitized to	Strip	Reactions to Substances Added to Bath in Order Given													
54	20 mg. 228B	1	0.5 cc. B.S.	−	1 mg. 230S	++	12 mg. 228B	+	10 mg. 230S	−	12 mg. 228B	−				
		2	0.5 cc. B.S.	−	1 mg. 228B	++	10 mg. 230S	−	12 mg. 228B	−						
		3	1 mg. 230S	+	12 mg. 230S	−	12 mg. 228B	−								
		4	1 mg. 230S	++	12 mg. 228B	+										
56	20 mg. 228B	1	0.005 mg. 230S	−	0.01 mg. 230S	−	0.1 mg. 230S	+	1.0 mg. 230S	+	10 mg. 230B	−	10 mg. 228B	−		
		2	0.001 mg. 228B	−	0.005 mg. 228B	−	0.01 mg. 228B	−	0.1 mg. 228B	+	1.0 mg. 228B	+	10 mg. 228B	−[1]		
58	20 mg. 228B	1	10 mg. 228B	++	10 mg. 228B	−	10 mg. 230S	−								
		2	10 mg. 230S	++	10 mg. 228B	−										
59	20 mg. 230 S	1	500 mg. S.S.S.	++	200 mg. S.S.S.	−	10 mg. 230S	−	45 mg. 247S	−						
		2	500 mg. S.S.B.	++	10 mg. 230S	−										
		3	10 mg. 230S	++	10 mg. 230S	−	500 mg. S.S.B.	−								
60	20 mg. 230S	1	0.5 cc. S.S.	−	10 mg. 230S	++	10 mg. 230S	−	10 mg. 228B	−						
		2	10 mg. 230S	++	10 mg. 230S	−	500 mg. S.S.B.	−								
61	20 mg. 230S	1	0.5 mg. 228B	++	10 mg. 230S	−										
		2	1.0 mg. 228B	++	10 mg. 230S	−	10 mg. 228B	−								
		3	1.0 mg. 230S	+	10 mg. 228B	−	10 mg. 230S	−								
Normal		1	10 mg. 228B	−	10 mg. 230S	−										

Note: B.S.=Beef serum; S.S.=Sheep serum; S.S.B.=Saline suspension of beef anterior lobes; S.S.S.=Saline suspension of sheep anterior lobes.
[1] Followed by a negative reaction to 10 mg. of 230S.

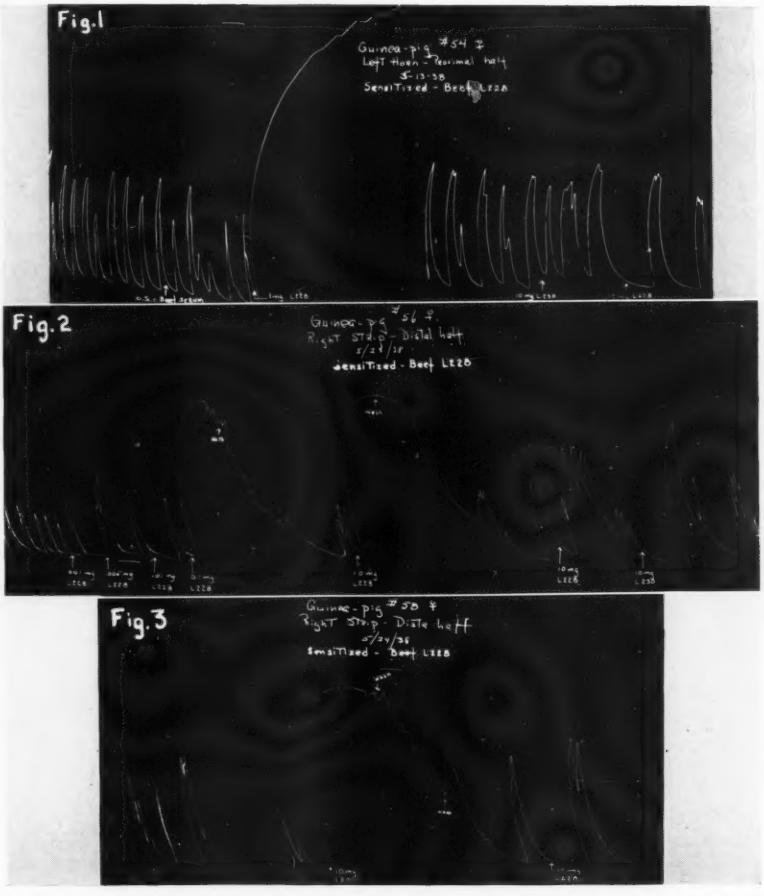

Fig. 1. DALE REACTION IN UTERINE STRIP 2 OF GUINEA PIG 54 SENSITIZED TO BEEF MAMMOTROPIN (228). Insensitive to 0.5 cc. beef serum, reactive to 1 mg. beef mammotropin and then desensitized to 10 mg. sheep (230) and beef mammotropin. Fig. 2. DALE REACTION IN UTERINE STRIP 1 OF GUINEA PIG 56 SENSITIZED TO BEEF MAMMOTROPIN (L 228). No reaction was obtained until the 0.1 mg. dose of beef mammotropin. A second reaction to 1.0 mg. was followed by desensitization. Fig. 3. DALE REACTION IN UTERINE STRIP 2 OF GUINEA PIG 58 SENSITIZED TO BEEF MAMMOTROPIN (228). A complete response was obtained to 10 mg. of sheep mammotropin (230), followed by desensitization even to the sensitizing antigen (228).

Strips 1 and 2 of guinea pig 61 reacted to 0.5 and 1 mg. respectively of BM and were subsequently found insensitive to SM, the preparation used in sensitizing the animal. The uterus of a young normal guinea pig was found insensitive to 10 mg. doses of both SM and BM as well as the crude saline suspensions.

To sum up the results with the uterine reactions it may be said that the beef and sheep mammotropic preparations used: *a*), contained no detectable amounts of the serum proteins of the animals from which they were obtained;

b), contained no detectable posterior lobe oxytocic principle; *c*), induced no change in the contraction rhythm of normal or desensitized uteri; *d*), caused complete Dale reactions in doses of 10 mg. or less followed by desensitization in animals sensitized to either; *e*), were probably not altered antigenically, in preparation; *f*), are equally good and indistinguishable antigens.

Serologic studies. In order that serologic and antihormone studies could be carried out, antisera against beef and sheep mammotropin were produced in rabbits. Injections of 40 mg. (2 cc. of a 2% solution) of mammotropin were made subcutaneously on 4 consecutive days of the week with no injections on the following 3 days. Four to 6 weeks of such treatment usually brought about a skin hypersensitivity and the appearance of high, though variable,

Fig. 4. Showing equally severe Arthus reactions to 1 mg. of sheep mammotropin (right upper quadrant) and 1 mg. of beef mammotropin (left upper quadrant) given intradermally in 0.25 cc. to rabbit 17 sensitized to sheep mammotropin.

titres of serum antibodies. Two male rabbits, *27* and *28*, were injected with BM and 2 males, *25* and *26*, and 3 females, *14*, *15* and *17*, were given SM. Males *2* and *4* were injected with beef and sheep serum respectively, and normal, uninjected virgins served as controls.

After a month of treatment the animals were bled from the heart and the serum obtained for precipitin, complement fixation, and antihormone tests.

Precipitin tests. In carrying out the precipitin tests, 0.1 cc. of clear, undiluted serum was layered beneath 0.5 cc. of dilutions of BM and SM. The organic weights of the antigens were known and therefore instead of giving the greatest dilution of antigen at which a test was positive, the calculated weight of antigen in that tube is listed. When the tests were positive, distinct rings were always apparent immediately at room temperature, but a final reading was always made after 30 minutes incubation at 37° C. To check our previous finding that the mammotropic preparations were free from serum proteins, each anti-mammotropin serum was tested against 1:10 and greater dilutions of beef and sheep sera, and in no instance was a positive precipitin test observed. Furthermore, rabbit antiserum capable of detecting

beef serum in a dilution of 1:10,000 gave no precipitin test when layered under beef mammotropin (0.001–1 mg.), nor did a 1:10,000 antiserum against sheep serum detect any sheep serum in preparations of sheep mammotropin (0.001 to 1 mg.).

Table 4 contains the results of parallel precipitin tests carried out with 5 anti-SM sera, and 2 anti-BM sera. The figures given represent the smallest amount of antigen in gamma giving a positive ring test with 0.1 cc. of antiserum. In 15 of the 21 tests listed BM and SM were indistinguishable, and of the other 6 tests only 3 suggested species-specificity. Thus the evidence obtained was definitely against specificity (18 out of 21 cases), as far as these two species were concerned. In 5 of the 6 cases of discrepancy, the difference amounted to 100% (or 1 tube), and it is well known that that amount of error is inherent in the ring-test, because of the difficulty in reading the end-

TABLE 4. MINIMAL AMOUNT OF BEEF (BM)+SHEEP (SM) MAMMOTROPIN IN γ DETECTABLE IN PRECIPITIN RING TESTS WITH 0.1 CC. OF ANTI-SERUM

Rabbit Serum	3-18-38		3-24-38		4-9-38		4-21-38	
	BM	SM	BM	SM	BM	SM	BM	SM
14 anti-SM	0.2	0.2	0.8	0.8	3.0	1.5		
15 anti-SM	0.1	0.1	0.4	0.2	0.1	0.1	0.1	0.1
17 anti-SM	0.2	0.2	0.2	0.2	0.4	0.8	0.8	0.8
25 anti-SM			1.0	2.0	0.8	0.8		
26 anti-SM			1.0	1.0	0.1	0.1	0.4	0.4
27 anti-BM			0.2	0.2	0.8	0.2	0.8	0.8
28 anti-BM					0.4	0.8	0.8	0.8

Note: Injections of animals 14, 15, and 17 were stopped just preceding the 3-18-38 bleeding. Injections of animals 25, 26, 27 and 28 were stopped just preceding the 3-24-38 bleeding.

point. The variation in titre of an individual animal seemed to depend on the time elapsing after the injections were stopped. Tests carried out later than those listed showed that the titres of all animals were gradually decreasing—a finding consistent with what is known about hypersensitive animals, and one that may also be explained by a possible, slow liberation of the animal's own mammotropic hormone.

Complement fixation tests. The complement fixation technic used, was essentially that of Kolmer, and all tests were rigidly controlled; 0.5 cc. (20γ) of the same antigens employed in 'immunization' was used in each test. 1.0 cc. of dilutions of pooled titrated guinea pig complement and 0.5 cc. of dilutions of antisera (inactivated at 56° C. for 20 minutes) were also added and the mixture left overnight in the icebox. The hemolytic system (consisting of 0.5 cc. hemolysin titrated in the presence of the dilution of antigen used in this test and 0.5 cc. of a 2% sheep cell suspension) was then added and the tubes incubated for 1 hour in the 37° C. water bath. Table 5 contains the results of parallel tests made with identical amounts of beef and sheep mammotropin. All of the sera (except those of normal rabbits) caused some degree of complement fixation, and in 4 of 7 parallel tests it was not possible to distinguish between heterologous and homologous antigens. Of the 3 other sets of tests, 2 (25 and 26) indicated that more complement was

fixed in the presence of homologous than heterologous antigen, while 1 (*15*) indicated the opposite. As with the precipitin tests, it was not possible on the basis of the majority of the complement fixation tests to distinguish between the beef and sheep mammotropic preparations used in these studies.

Antihormone tests in pigeons. In testing the sera of normal rabbits, as well as those of rabbits injected with beef or sheep serum, and BM or SM for their antimammotropic effect, 5 daily intrapectoral injections of the sera were made in pigeons, beginning on the day before the first hormonal injection and continuing on the 4 days on which the hormone was administered

TABLE 5. DILUTIONS OF ANTISERA AT WHICH COMPLEMENT FIXATIONS OCCURRED IN THE PRESENCE OF 20γ OF BEEF OR SHEEP MAMMOTROPIN

Rabbit Serum	BM	Fixation	SM	Fixation
Normal *1*	1:13	−	1:13	−
14 Anti-SM	1:26	++++	1:26	++++
	1:52	++	1:52	++
15 Anti-SM	1:104	++++	1:104	++++
	1:208	++++	1:208	+++
17 Anti-SM	1:104	++++	1:104	++++
	1:208	+++	1:208	+++
Normal *2*	1:20	−	1:20	−
25 Anti-SM	1:20	+	1:20	++++
	1:40	−	1:40	+++
26 Anti-SM	1:40	++++	1:40	++++
	1:80	−	1:80	+++
27 Anti-BM	1:40	++++	1:40	++++
	1:80	+++	1:80	+++
28 Anti-BM	1:40	++++	1:40	++++
	1:80	+++	1:80	+++

Note: The greatest dilution at which complement fixation was observed is listed.

subcutaneously in the sacral region (the intramuscular injection of the serum being given just prior to the subcutaneous injection of hormone). The squabs were always killed 24 hours after the fourth injection of hormone. Only the reactive areas of the crop were weighed, and unit evaluation made according to a curve previously worked out. Since table 6 contains most of the unit values for crops varying from the control weight of about 1500 mg. to those around 3500 (the 'sensitive' range) it will be unnecessary to reproduce the curve here. The figures given represent the average weight of crop sacs for the 3 birds used on each test. The birds were all Silver Kings, 4 to 5 weeks from hatching, weighing 400 to 500 gm. Considerable variation was encountered in reactivity of individual birds and this is partially corrected by using 3 birds for each test. The observed crop weights in table 6 are translated into apparent unit values which show how many of the injected units escaped neutralization, and therefore functioned in causing crop muco-

sal hyperplasia. The difference between the number of units injected and the number that apparently functioned is listed as units neutralized. The sera used for antihormone tests were titred for precipitins against the hormonal preparation used, and it will be observed that most of the tests have been done with sera that detected 1γ or less of mammotropin, and that it usually required 2.5 cc. of a total dose to show definite neutralization. Both BM and SM were neutralized to the same degree, and a saline extract or emulsion of

TABLE 6. MAMMOTROPIN-NEUTRALIZING POTENCIES OF THE STRONGER ANTISERA AND THEIR PRECIPITIN TITRES

Rabbit serum	Date bled	Precipitin titre (γ)	Serum injected cc.	Mammotropin	Units injected	Average weight 3 crops-mg.	Apparent unitage	Units neutralized
—	—	—	—	L 228 B	4.0	2800	4.0	—
1 Normal	—	—	2.5	L 228 B	4.0	2866	4.2	—
15 Anti-SM	3/24	0.2	2.5	L 228 B	4.0	1700[1]	1.0	3.0
15 Anti-SM	4/9	0.1	0.05	L 228 B	4.0	3033	5.2	Augmentation
15 Anti-SM	4/9	0.1	0.1	L 228 B	4.0	2633	3.5	0.5
15 Anti-SM	4/9	0.1	0.2	L 228 B	4.0	2166	2.1	1.9
15 Anti-SM	4/9	0.1	0.5	L 228 B	4.0	1600[1]	1.0	3.0
15 Anti-SM	4/9	0.1	1.0	L 228 B	4.0	1666[1]	1.0	3.0
15 Anti-SM	4/9	0.1	2.5	L 228 B	4.0	1500[2]	0.0	4.0
—	—	—	—	L 231 S	2.0	2100	2.0	—
15 Anti-SM	3/11	0.2	2.5	L 231 S	2.0	1500[2]	0.0	2.0
15 Anti-SM	5/6	0.2	2.5	L 241 S	6.0	2333	2.6	3.4
—	—	—	—	L 241 S	6.0	3200	6.0	—
—	—	—	—	L 250 S[3]	8.0	3650	8.0	—
15 Anti-SM	5/6	0.2	2.5	L 250 S	8.0	3100	5.5	2.5
15 Anti-SM	5/31	0.2	3.0	L 247 S	7.0	2333	2.6	4.4
—	—	—	—	L 247 S	7.0	3466	7.0	—
1 Normal	—	—	3.0	L 247 S	7.0	3466	7.0	—
17 Anti-SM	5/31	0.2	3.0	L 247 S	7.0	2533	3.5	3.5
17 Anti-SM	3/11	0.2	2.5	L 231 S	2.0	1650[2]	0.0	2.0
17 Anti-SM	3/18	0.2	2.0	L 228 B	4.0	1733[1]	1.0	3.0
17 Anti-SM	3/24	0.2	2.0	L 228 B	4.0	2033	1.9	2.1
17 Anti-SM	4/9	0.4	2.0	L 228 B	4.0	2100	2.0	2.0
17 Anti-SM	4/21	0.8	2.0	L 228 B	4.0	2333	2.6	1.4
14 Anti-SM	4/9	3.0	2.5	L 228 B	4.0	3400	7.0	Augmentation
14 Anti-SM	3/18	0.2	2.5	L 231 S	2.0	1550[2]	0.0	2.0
25 Anti-SM	3/24	2.0	2.5	L 228 B	4.0	3333	6.1	Augmentation
26 Anti-SM	5/31	0.8	3.0	L 247 S	7.0	3666	8.0	Augmentation
26 Anti-SM	4/9	0.1	2.0	L 228 B	4.0	1500[1]	1.0	3.0
27 Anti-BM	3/24	0.5	2.5	L 228 B	4.0	2266	2.4	1.6
27 Anti-BM	4/9	0.8	2.5	L 228 B	4.0	2266	2.4	1.6
28 Anti-BM	4/9	0.4	2.5	L 228 B	4.0	1966	1.8	2.2

[1] 1 crop negative; other 2 just positive.
[2] All crops negative.
[3] Saline emulsion.

untreated fresh sheep pituitaries was likewise neutralized by an anti-mammotropin serum. In a few instances when the antiserum was of a weak titre an apparent augmentation of the hormonal effect was seen, but with sera capable of detecting below 1γ of hormone, this occurred only once (R 26, 5/31), (see also 0.08 cc. level of R 15, 4/9).

The mechanism of the antihormonal action of these antisera is quite unknown to us. It has been observed that hormonal-antiserum mixtures that have been incubated together for 1 hour at 37° C., and then kept at ice-box temperature during the 4-day injection period give, if anything, an augmentary effect and certainly not a neutralizing one. Besides the possibility that a

sufficient amount of antiserum may prevent the fixation of mammotropin by the crop epithelium, one must consider also the likelihood of increased renal elimination, since it is known that this hormone is normally excreted by the kidney.

The Arthus phenomenon. Since the rabbits were 'immunized' by subcutaneous injections, it became apparent, especially after the third week, that hypersensitivity had developed to the mammotropin, because severe skin reactions occurred. That the extracts themselves were not primarily toxic was shown by the fact that the earlier injections caused no skin reactions; nor did the same extracts when tested on new animals show any skin toxicity. At the end of the course of injections for antisera, 3 of the rabbits were skin-tested with homologous and heterologous antigens in order to obtain further information regarding species-specificity of mammotropin. Rabbits *15* and *17* sensitized to sheep mammotropin, rabbit *28* sensitized to beef mammotropin, rabbit *2* sensitized to beef serum, and a normal rabbit were each injected intradermally as follows: 0.25 cc. (1 mg.) of sheep mammotropin in the right upper quadrant of the shaven ventral skin; 0.25 cc. (1 mg.) beef mammotropin in the left upper quadrant; 0.25 cc. sheep serum in the right lower left quadrant, and 0.25 cc. of beef serum in the left lower quadrant. The normal rabbit showed no reaction other than the slight hyperaemia that may attend the primary injection of foreign protein. The animal sensitized to beef serum showed a positive Arthus reaction to beef serum only. Rabbits *15* and *17* showed equally severe reactions (necrosis, slough and scab formation) to BM and SM and only a slight hyperaemia at the sites of serum injection. (See fig. 4 for equally positive Arthus reactions to BM and SM in rabbit *17*, sensitized to BM). Rabbit *28* sensitized to BM showed equally severe reactions to BM and SM, and negative reactions to beef and sheep sera.

By use of the Arthus phenomenon, then, it was impossible to distinguish between preparations of beef and sheep mammotropin, equally severe skin reactions being obtained with the antigen used in preparing the rabbit and the heterologous one.

DISCUSSION

That we have worked with an antigenic substance that can be prepared from beef or sheep pituitaries, has been shown by all of the commonly used immunologic methods. Using the active and passive anaphylactic reactions, the Dale and Arthus reactions and the precipitin and complement fixation serologic tests, it has not been possible to distinguish beef mammotropic preparations from those made in like manner from sheep pituitaries. It is possible, but not probable, that breaking these proteins down into their amino acid components will reveal any great chemical differences since the immunologic methods, especially the Dale reaction (5), are chemically discriminating to a high degree.

It is not assumed that we have worked with the pure mammary-stimulating hormone. The same results might conceivably have been obtained from mixtures of more than one species-non-specific protein. Even granting that we are dealing with a single protein, the final identification of the hormone

with that protein must await crystallization. Therefore it is only possible to say, at this time, that protein preparations identical antigenically, as well as with regard to their mammary and crop-stimulating potencies have been prepared from beef and sheep pituitaries.

That we may have altered two dissimilar proteins in such a way as to make them antigenically identical is conceivable, and more data must be gathered to decide this. At present it is difficult to rid our mammotropin of the other hormones as well as serum proteins without rather high concentrations of the only reagents used (HCl, NH_4OH and acetone). Against the likelihood of alteration by treatment is the finding that animals sensitized to purified mammotropin react to that substance as it exists in freshly made saline suspensions of the anterior lobe.

Analysis of our results from the standpoint of the recent antihormone revival is difficult because we cannot possibly satisfy all of the proponents of divergent theories. Influenced by an earlier study (6) and following the reasoning of Hektoen (7), we would be inclined to interpret the anti-hormonal effect observed in this work on the same basis as the other so-called 'immune' phenomena. Since optimal proportions of mammotropin and its precipitin form an insoluble aggregate in vitro, it is possible that with potent antisera, this also occurs in the passively 'immunized' pigeon injected with the hormone. If such an aggregate should be formed in the pigeon's blood stream, one is faced still with the difficulty of explaining why the bound mammotropin cannot act upon the crop, for we have injected subcutaneously into pigeons the precipitates, formed in vitro, and found that the hormone is still effective as shown by the crop reaction. In fact, on several occasions we have observed augmented reactions rather than inhibition from such mixtures, and have interpreted them as evidence of slow release of the hormone from the aggregate.

SUMMARY

Mammotropic preparations made from beef or sheep pituitaries were found to be antigenically indistinguishable as far as could be determined by the technics employed for active and passive anaphylaxis, Dale and Arthus reactions, precipitin and complement fixation tests.

The crop-stimulating effect of beef and sheep mammotropin was neutralized by injecting the pigeons with sufficiently high dosage of antimammotropin serum prepared in rabbits against either beef or sheep mammotropin.

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THE URINARY EXCRETION OF ANDROGENIC SUBSTANCE AFTER THE ADMINISTRATION OF TESTOSTERONE PROPIONATE TO THE IMMATURE MONKEY[1]

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As a part of an investigation of the intermediary metabolism of the naturally occurring androgens we are presenting in this communication a study of the androgenic substances excreted in the urine of immature monkeys after the administration of testosterone propionate.[2]

MATERIAL AND METHODS

Two immature male and 2 immature female *Macacus mulatta* monkeys were chosen as test animals. The males weighed 2.0 and 2.1 kg. respectively, the females 1.6 and 1.8 kg. One male and one female monkey, animals 1 and 4, each received single injections of 20 mg. of testosterone propionate on 2 occasions; after 22 days with no further treatment, a course of 17 injections of 20 mg. each, was given on alternate days over a period of 35 days. The other male and female monkeys, animals 2 and 3 received single injections of smaller amounts of hormone, 5 mg. of testosterone propionate, on each of 2 occasions and, after 22 days without treatment, a series of 14 injections of 5 mg. which were given on alternate days over a period of 30 days.

During the long period of continued injections the monkeys responded to the androgen in such a manner as to show that both the 5 and 20 mg. doses were sufficiently strong to evoke characteristic responses to androgens. In both males the testes descended to the scrotum (1) from the bilateral cryptorchid position of the immature monkey (2). In the females the clitoris became greatly hypertrophied and developed a glans (3), and in both males and females there appeared a redness extending from the perianal region to the base of the tail to the pubis and to other areas of sexual skin (3, 4). In female 4 the redness was only slight and limited to the perianal region, although she received the large doses of 20 mg. In view of this poor sexual skin response to the androgen and a somewhat hairless appearance, tuberculin tests were made on this animal and on the others since they had been exposed to her. All but animal 2 had positive reactions and at autopsy active pulmonary tubercular lesions were found. The involvement of the lungs was most extensive in female 4.

The peanut oil vehicle for the tetosterone propionate was standardized at 1 cc. per dose since the type and amount of oil influences the effectiveness of the hormone (5). All injections were made intramuscularly in the arm or leg. When injections were given over long periods of time, each limb was chosen in turn so that no one limb received an injection more often than every fourth time. Fifteen urine samples

[1] Aided by the Fluid Research Fund, the General Education Board of the Rockefeller Foundation, the Committee on Scientific Research of the American Medical Association, and the International Cancer Research Foundation.

[2] Testosterone propionate was furnished through the courtesy of the Ciba Pharmaceutical Company, Inc., under the trade name of perandren.

preserved with toluene were collected from each of the monkeys over a period of 135 days. This represented complete 24-hour excretions over the entire observation period. Each urine sample was quantitatively extracted and purified according to a method previously described (6). The androgenic activity of the urine extracts was determined by use of the day-old chick's comb (7) and expressed in terms of international units (I.U.), which is the androgenic activity evoked by 0.1 mg. of androsterone. Fifteen chicks were used in each of 40 assays. In the remaining 16 determinations, the amount of active material was so small that only 10 chicks were used for an individual assay.

RESULTS AND DISCUSSION

No androgenic activity was detected in the urine of any of the 4 immature monkeys before testosterone propionate was injected. If androgens are

TABLE 1. EXCRETION OF ANDROGENIC SUBSTANCE AFTER ADMINISTRATION OF TESTOSTERONE PROPIONATE

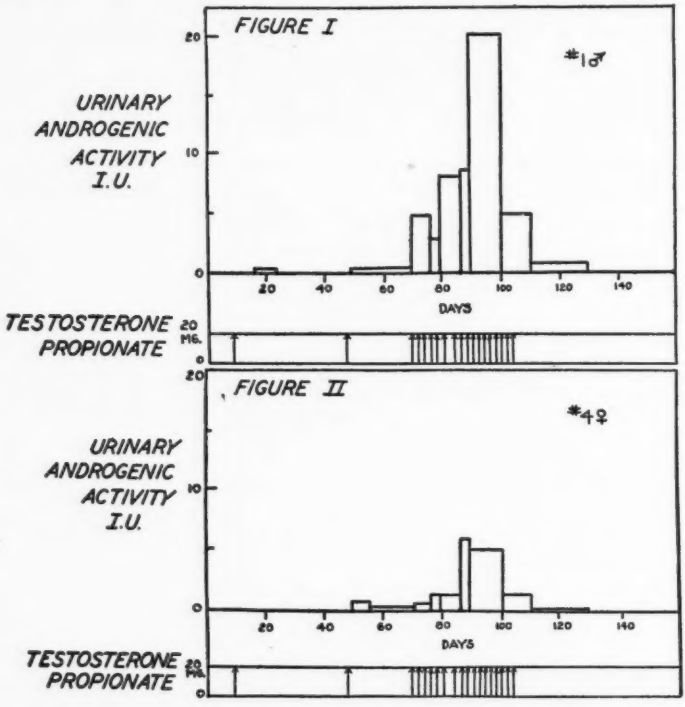
Monkey and Sex	No. injections	No. days over which injections were made	Amount of testosterone propionate injected		Androgenic substance excreted I.U.	Androgenic substance excreted calculated as androsterone %
			Calculated as testosterone propionate mg.	Calculated as testosterone mg.		
1 ♂	1	1	20	16.7	2.4	1.2
	1	1	20	16.7	7.5	4.5
	17	35	340	283.3	451.0	15.9
2 ♂	1	1	5	4.2	<7.6	<1.8
	1	1	5	4.2	<5.8	<1.4
	14	30	85	71.0	55.6	7.8
3 ♀	1	1	5	4.2	6.8	1.6
	1	1	5	4.2	7.0	1.7
	14	30	70	58.5	63.9	11.0
4 ♀	1	1	20	16.7	9.6	5.7
	1	1	20	16.7	10.7	6.4
	17	35	340	283.3	154.0	5.4

present in these urines, they are in concentrations of less than 0.05 I.U. per day.

The excretion of urinary androgenic material following the administration of various amounts of testosterone propionate is presented in table 1 and figures 1 to 4. The data in table 1 are calculated as though the injected testis hormone is excreted as the urinary hormone, androsterone. Although testosterone is converted in the human body to androsterone (8, 9), one must also recognize the possibilities of testosterone, dehydroisoandrosterone or undescribed androgens being present in these urines. If the excreted androgen be testosterone, the percentage excreted would be one-sixth of that listed in the table, or if dehydroisoandrosterone, 3 times greater. The androgenic activity in the urine may be due, however, to a mixture of 2 or more substances.

When single doses of 20 mg. of testosterone propionate were administered to monkeys 1 and 4, the excreted androgenic material calculated as androsterone represented between 1.2 and 6.4% of the amount administered. In the same animals, when 340 mg. of testosterone propionate was adminis-

tered over a period of 35 days, 15.9% was excreted in the case of the immature males and 5.4% in the case of the immature female. During the chronic injections, the daily urinary excretion of androgens of the male monkey rose to as high as 23 I.U. a day for a period of 11 days (fig. 1), whereas in the female the highest level of excretion was 7.7 I.U. (fig. 2) during a 3-day period. The excretion of androgens persisted for 25 days



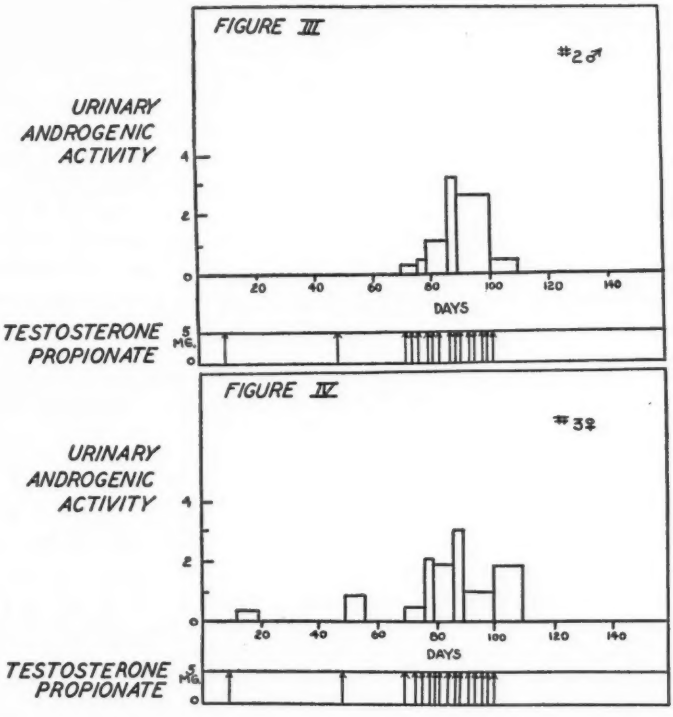
THE EXCRETION OF ANDROGENIC SUBSTANCES AFTER THE ADMINISTRATION OF TESTOSTERONE PROPIONATE. Fig. 1. Male weighing 2.1 kg. given doses of 20 mg. Fig. 2 Female weighing 2.0 kg. given doses of 20 mg. Note that the level of androgenically-active material in the urine is increased following the administration of male hormone substance to the animals and that this increase is greater in the latter part of the period of continued injections than in the first few days.

after the cessation of chronic injections, a fact which may be correlated with the continued presence of the injected oil solution in the tissue for a long period of time.

In monkeys 2 and 3, which received amounts of 5 mg., the activity of the substances excreted in the urine, if calculated as androsterone, was between 1.7 and $<1.4\%$ (table 1) of the administered testosterone propionate. When injections were continued for a 30-day period, 85 mg. to the male animal, 2, and 70 mg. to the female, 3, a greater percentage excretion was found, 7.8% for the male and 11.0% in the female. After the cessation of chronic injections, the excretion of androgenic material continued for 10 days (fig. 3, 4). It is interesting that the immature male monkey receiving continued

injections of 5 mg. on alternate days, had a urinary level of androgenic activity that was of the same magnitude as that found in the case of the normal adult male monkey (10).

The data of female 4 are not considered to be conclusive inasmuch as the animal became emaciated during the experiment and was shown at autopsy to be in an advanced stage of tuberculosis. Excluding this animal there appears to be a higher level of androgenically-active urinary substances during



THE EXCRETION OF ANDROGENIC SUBSTANCES AFTER THE ADMINISTRATION OF TESTOSTERONE PROPIONATE. Fig. 3. Male weighing 1.8 kg. given doses of 5 mg. Fig. 4. Female weighing 1.6 kg. given doses of 5 mg.

long-continued treatment than after a single injection. This represents in all 3 animals between 5 and 6 times the percentage recovery after single injections.

With the continuation of injections there is evidence of a higher level of androgenic substance in the urine, at least for a limited time. In male 1 average daily excretion in the first 6 days was 5 I.U., during the next 12 days 7 I.U., and in the following 10 days 23 I.U. In male 2 the average daily excretion was 0.4 I.U. in the first 6 days, 1.4 I.U. in the next 12 days, 2.4 I.U. in the following 10 days. In female 3 the average daily level was 0.5 I.U. in the first 6 days, 2.2 I.U. in the next 12 days and 1.2 I.U. in the last 10 days. Comparing the excretion, then, in the first 6 days with that of the following

18 days the percentage recovered was 2½ times greater in animal 3, 2½ in animal 1, and 4½ in animal 2.

Kochakian (11) reported that after subcutaneous administration of urinary male hormone extracts and synthetic androstenedione to men, rabbits, and dogs, the recovery in the urine in terms of activity was from 2 to 6% of the injected material. It is difficult to compare this with our findings, since he studied the androgen excretion after the administration of urinary extracts and androstenedione, while we investigated testosterone. In experiments with adult male and female rats, from 1 to 3% (on the basis that the urinary androgen is androsterone) of injected testosterone could be accounted for in the urine after the administration of 10 to 25 mg. of hormone (12).

In a man with deficient male hormone secretion who was injected intramuscularly with 600 mg. of testosterone propionate in doses of 20 mg. daily for 30 days, the recovered material, if calculated as androsterone, was 41.4% (13). This is almost 3 times higher than the excretion found in the 2-kg. monkey 1 when 340 mg. was administered over a period of 35 days. This greater urinary output of androgens in the case of the man may be a result of the quantity of testosterone exceeding a possible kidney threshold or of a species difference in the intermediary metabolism of the male sex hormone.

SUMMARY

The quantitative excretion of androgenic substances in the urine after the intramuscular administration of testosterone propionate has been investigated in immature male and female monkeys. If the percentage of recovered androgens is calculated on the basis that testosterone is converted to and excreted as androsterone, the administration of 20 mg. of hormone in a single dose results in the recovery of between 1.2 and 6.4%. After a single dose of 5 mg. between <1.4 and 1.7% is excreted. When 340 mg. in divided doses was administered on alternate days for over a month, between 5.4 and 15.9% was found in the urine. After the injection of 70 and 85 mg. in 30 days, 7.8 and 11%, respectively, were present in the urine.

If the urinary androgenic substances were testosterone the percentage recovered would be one-sixth of the above figures, if dehydroisoandrosterone 3 times greater.

With continuation of the administration of testosterone, the amount of androgenic substance in the urine is increased over that present during the first days of injection.

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THE URINARY EXCRETION OF ESTROGENIC SUBSTANCES AFTER THE ADMINISTRATION OF TESTOSTERONE PROPIONATE[1]

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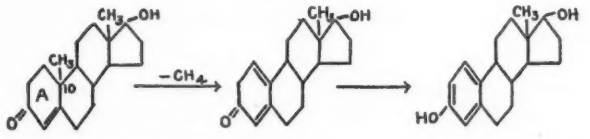
TRANSFORMATION of testosterone, the male sex hormone isolated from bull testis tissue, into a substance possessing estrogenic activity, has been claimed by Steinach and Kun (1) to occur in men. No such transformation was found by Kochakian (2) in the dog, or by Dorfman (3) in the rat. Steinach and Kun were able to increase the urinary excretion of estrogenic material in the urine of men after the injection of testosterone propionate. In one case they report an increase in estrogenic activity from approximately 36 to 1200 R.U. per liter after the injection of 1 gm. of testosterone propionate during a period of 6 weeks. Their assays for estrogenic activity were made on the crude benzene extracts so that it is difficult to say what the nature of the excreted estrogenic substance may be. Further, the possibility exists that this activity may be due to androgens such as androstenediol or dehydroisoandrosterone, which are also capable of producing estrogenic effects.



TESTOSTERONE ESTRADIOL

In castrate and normal dogs Kochakian was unable to demonstrate an increase in urinary estrogenic activity after the injection of testosterone or its benzoate, oxime, or acetate in amounts ranging from 45 to 238 mg. Similarly, no increased excretion of urinary estrogens was detected after the administration of from 0.2 to 25 mg. of testosterone to adult male or female rats (3).

The suggestion has been made (4), however, that the testosterone might be converted to naturally-occurring phenolic estrogens like estradiol and estrone by demethylation in position 10 and subsequent rearrangement in ring A. No direct proof of this conversion has been described. The present study is an investigation of *a*), a possible increase in the amount of estrogens

[1] Aided by grants from the General Education Board of the Rockefeller Foundation, the International Cancer Research Foundation, and the Fluid Research Fund of Yale University School of Medicine.

excreted after the administration of testosterone propionate[2] to men and to immature monkeys, and *b*), (if more estrogenic substances are excreted) whether these estrogens are phenolic such as estradiol, estrone, and estriol.

MATERIAL AND METHODS

The patients were 2 men, described previously (5, 6), aged 35 (*case 1*) and 27 (*case 2*) years. They exhibited organic and functional evidence of deficient testicular secretion (5–8).

Case 1 received large amounts of testosterone propionate, first intramuscularly and later orally. Two collections of urine, one for 4 days, one for 1 day, served as control samples. Daily intramuscular injections of 20 mg. of testosterone propionate dissolved in 1 cc. of peanut oil were given for 30 consecutive days. During this period 14 representative 24-hour samples were collected and during the 37 days following cessation of hormone treatment, 6 24-hour samples were collected (table 1).

TABLE 1. URINARY ESTROGENIC ACTIVITY AFTER INTRAMUSCULAR ADMINISTRATION OF TESTOSTERONE PROPIONATE TO THE HUMAN MALE

Day of treatment	Urinary estrogenic activity, I.U./24 hr.	Day after stopping treatment	Urinary estrogenic activity, I.U./24 hr.
0	10		
0	7		
1	8	3	35
3	30	8, 9	23
4	50	16	18
5, 6	30	30	10
8	36	37	10
9, 10	50		
11	25		
12, 13, 25, 26, 27	50		

Case 1 received intramuscular injections of 20 mg. of testosterone propionate in 1 cc. of peanut oil daily for 30 days.

In another experiment involving oral administration of powdered crystalline testosterone propionate, he received 60 mg. daily for a period of 15 days, followed by ingestion of 120 mg. daily for 7 days (table 2). Two 24-hour urine samples were collected, one during the period when 60 mg. was taken daily, the other when 120 mg. was taken daily.

Case 2 ingested 60 mg. of testosterone propionate daily for 14 days, during which time 9 24-hour samples were collected.

The daily dose for each patient was divided into 16 parts, which were taken at hourly intervals.

In addition to the human experiments, 2 male and 2 female immature *Macacus mulatta* monkeys were given intramuscular injections of testosterone propionate as indicated in table 3. Complete urine collections for 135 days were made by keeping the animals in metabolism cages. The weight of the monkeys ranged from 1.6 to 2.1 kg. A few weeks after the conclusion of the experiment autopsies were performed on both of the females and one of the males, with the finding of tuberculous lung lesions in all but animal 2.

All urine samples were extracted with benzene in a continuous extractor by a

[2] Testosterone propionate was furnished through the courtesy of the Ciba Pharmaceutical Company, Incorporated, under the trade name perandren.

method previously described (9). In order to measure the phenolic estrogens the alkali soluble fraction of the urine extracts were assayed for estrogenic activity. Adult spayed female mice served as test animals, 10 or more being used for each assay. Estrogenic assays are expressed in terms of international units (I.U.), which is the estrogenic activity evoked by 0.1 microgram of estrone.

RESULTS AND DISCUSSION

The excretion of estrogenic substances after the intramuscular administration of testosterone propionate to *case 1* is listed in table 1. The daily level of urinary estrogens was increased from the control value of 10 I.U. to 30 I.U. on the 3rd day. The average excretion for 7 assays representing 13 days of collection between the 3rd and 27th days was 42 I.U. per day. This represents an increase of approximately 500% above the level found prior to treatment with testosterone. During the period following the injections of the hormone the level of urinary estrogens fell until at 30 to 37 days following the cessation of treatment the values reached those found before treatment.

In contrast to the effect noted after intramuscular injections, oral administration of 6 times the amount given intramuscularly to the same patient resulted in no increase in urinary estrogens (table 2). In *case 2* practically

TABLE 2. URINARY ESTROGENIC ACTIVITY AFTER ORAL ADMINISTRATION OF TESTOSTERONE PROPIONATE TO THE HUMAN MALE

Case	Day of Treatment	Urinary Estrogenic Activity I.U./24 hours
1	0	10
	0	10
	3	10
	22	12
2	0	12
	0	12
	1	16
	2	11
	4	14
	6	12
	7, 9, 11, 12	13
	13	20

Case 1 ingested 60 mg. of testosterone propionate daily for 15 days, 120 mg. of testosterone propionate daily for the next 7 days.

Case 2 received 60 mg. of testosterone propionate orally for 14 days.

negative findings were also found after oral administration of the hormone. The average of 6 assays representing 9 of the first 13 days of treatment was 13.9 I.U. per day as compared to 12 I.U. found before treatment.

In the male and female immature monkeys no estrogenic activity was detected in samples of urine taken before treatment. If activity is present in the urine of these animals, the quantity is less than 0.8 I.U. per day. Intramuscular administration of testosterone propionate caused some increase in urinary estrogens (table 3). Values obtained in 2 of the assays are anomalous. After the first injection of 20 mg. of hormone in monkey 1, eleven I.U. per day was excreted for 7 days; when the above treatment was repeated a

TABLE 3. URINARY ESTROGENIC ACTIVITY AFTER INTRAMUSCULAR ADMINISTRATION OF TESTOSTERONE PROPIONATE TO THE MONKEY

No. of monkey and sex	Weight	Amount of testosterone propionate injected	Assay	No. of days of urine collection	Urinary estrogenic activity I.U./24 hr.
	kg.	mg.			
1 ♂	2.1	0	1	9	<0.8
		20	2	7	11.0
		20	3	7	<1.3
		340[1]	4	40	1.3
2 ♂	2.0	0	5	9	<0.8
		5	6	7	<1.1
		5	7	7	<1.1
		85[2]	8	30	<1.5
3 ♀	1.8	0	9	9	<0.8
		5	10	7	1.4
		5	11	7	<0.9
		70[2]	12	30	<1.0
4 ♀	1.6	0	13	9	<0.8
		20	14	7	2.7
		20	15	7	1.5
		340[1]	16	40	0.5

[1] Administered in 17 equal doses over a period of 35 days.
[2] Administered in 14 equal doses over a period of 30 days.

second time, less than 1.3 I.U. per day was detectable during the 7-day period; when 340 mg. of testosterone was administered over a period of 35 days the value of estrogens rose to only 1.3 I.U. per day. The second irregularity in the results was seen in monkey 4 in which, after single injections of 20 mg. of hormone on 2 occasions, 2.7 and 1.5 I.U. per day respectively was found, but during a later experimental period when 340 mg. was injected over 35 days, only 0.5 I.U. per day was found. This failure during the latter part of the experiment to excrete appreciable quantities of estrogens is probably correlated with poor health and a marked decrease in the response of the animal to the androgen as seen in the disappearance of the redness in sexual skin and nasal turbinate areas. The animal became emaciated during the latter part of the experiment and extensive tuberculous lesions were found at autopsy.

With the exception of assay 4 in animal 1 and assay 16 in animal 4, the data regarding increase in estrogens indicates that there is little if any increase following repeated injections of 5 mg. in the male or female monkey, but a definite increase when 20 mg. is given in single or repeated doses to either male or female animals (table 3).

When a single dose of 5 mg. of testosterone propionate was injected, as was the case in monkeys 2 and 3, estrogenic activity was detected in only one instance, that is, in the urine of monkey 3, in which 1.4 I.U. per day was found. Similarly, during the period of chronic treatment, when 85 mg. of hormone was administered over 30 days to monkey 2 and 70 mg. over the same period to monkey 3, no estrogenic activity was detected.

These data show an increase in urinary phenolic estrogens after the intramuscular administration of testosterone propionate. As to the source of this phenolic estrogen, two possibilities must be recognized: *a*), the direct conversion of testosterone into a known phenolic estrogen as estrone, or into an undescribed phenolic estrogen; *b*), the gonadal or extragonadal production of a phenolic estrogen under the influence of testosterone. The quantitative relationship between amounts of administered testosterone and the increased amounts of urinary estrogenic activity may be examined in table 4 with regard to the possibility of direct conversion of testosterone

TABLE 4. CALCULATION OF PHENOLIC URINARY ESTROGENS AFTER ANDROGENIC TREATMENT OF CASE

Testosterone propionate administered (mg.)	20 t.p.= 16.7 t
Estrogens recovered in urine after treatment (I.U. 24 hrs.)	42
Estrogens recovered in urine before treatment (I.U. 24 hrs.)	7
Net increase in excretion of estrogens (I.U. 24 hrs.)	35
If excreted estrogen is estrone	
Net weight (mg.)	0.0035
% of weight of administered androgen	0.02
If excreted estrogen is estriol	
Net weight (mg.)	0.175
% of weight of administered androgen	1.0

into a phenolic estrogen such as estrone. When 20 mg. of testosterone was injected intramuscularly, only 0.02% of the administered male hormone could be recovered in the urine if the urinary estrogen were estrone (table 4). It is possible that the administered testosterone was converted to an undescribed phenolic estrogen which is less active than estrone and therefore the estrogenic activity would actually represent a greater weight of material and consequently a greater percentage of conversion. To test the second possibility, that is, the gonadal or extragonadal production of a phenolic estrogen under the influence of testosterone, it will be necessary to study the effect of testosterone in the castrated subject. Whatever the substances and however produced, the increased amount of estrogens obtained after the administration of androgens is suggestive that some portion of the urinary estrogen output of men under normal conditions may be due not alone to any direct production by the endocrine or other system, but is rather a phenomenon influenced by the presence of male hormone. Such a male hormone influence upon the estrogen output in men seems all the more probable in view of the low estrogen output when the urinary level of androgens is also low as in hypogonadal men (10) and in immature boys (11). It is interesting that during intramuscular injections of *case 1* the average level of estrogen excretion in the urine (42 I.U.) was within the lower limits of the average found in 4 normal men investigated by Gallagher and coworkers (9).

The fact that orally administered testosterone did not give rise to an increased estrogen excretion may be correlated with the fact that, although the administered substance was absorbed from the gastro-intestinal tract, it appeared to be very rapidly dissipated, for androgenically-active material appeared in large amounts in the urine (7). Clinically, phenomena accom-

panying states of deficient testicular secretion were not relieved by the oral administration of testosterone propionate (12), so adequate androgenic levels in the body tissues and fluids was not maintained by the orally-administered testosterone propionate. A large amount of testosterone can be absorbed from the gastrointestinal tract and rapidly eliminated without increased estrogen activity, whereas 1/6 of the oral dose if given intramuscularly in oil to the same patient resulted in more effective body levels and produced increased amounts of estrogens in the urine.

SUMMARY

After administration of the testis male hormone, testosterone propionate, to 2 men with deficient testicular secretion and to 4 immature *Macacus mulatta* monkeys (average body weight 2 kg.), the urine was examined with regard to change in estrogenic activity. Since all assays were made on the alkali-soluble fraction of the urine extract, the estrogenic substances determined were phenolic, hence chemically related to, if not known naturally-occurring estrogens like estrone or estriol.

In *case 1* of the men, intramuscular injection of 20 mg. of testosterone propionate resulted in a 500% increase in the estrogenic activity of the urine. Oral administration of 6 times this amount to the same patient, and of large quantities to the other man, gave no substantial increase in urinary estrogens. On the basis of previous studies, it is suggested that absorption from the gastro-intestinal tract and dissipated by the body is so effective that the influence of the androgenic substance is not as continued and effective as when testosterone propionate is given intramuscularly.

In the immature monkeys, the results of intramuscular injections of large amounts of testosterone propionate are suggestive of increased estrogen excretion.

Increased estrogenic activity of the urine following male hormone administration indicates that some portion of the female hormone output of normal men might be a result of the influence of male hormone.

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STUDIES ON DESOXY-CORTICOSTERONE[1,2]

A SYNTHETIC ADRENAL CORTICAL HORMONE

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IN 1937 STEIGER AND REICHSTEIN (1) announced the preparation of desoxy-corticosterone acetate (Δ^4-pregnene-21-ol-3, 20-dione, acetate) from stigmasterol. Recently, Reichstein (2) has succeeded in isolating desoxy-corticosterone from an extract of beef adrenal. In a previous publication (3) we have compared the efficacy of desoxy-corticosterone acetate (synthetic) and adrenal cortical extract in producing a retention of sodium and chloride in normal dogs and in maintaining the electrolyte balance of an adrenalectomized dog. In the present report we have studied the effectiveness of single daily subcutaneous injections of a solution of desoxy-corticosterone acetate in oil in maintaining adrenalectomized dogs fed a diet of low sodium and chloride content.

METHODS

Male dogs (approximately 10 kg.) were maintained in metabolism cages and fed a constant diet composed of 350 to 400 gm. of ground beef to which was added 50 cc. of irradiated evaporated milk.[3] The quantity of ground beef fed to different animals varied somewhat, being determined by the amount necessary to maintain an animal's weight under normal conditions. Once established, the quantity of meat given a dog was kept constant throughout the experiment. At the completion of each 24-hour period the animals were catheterized and weighed. Twenty-four-hour urine specimens were collected and preserved with toluene. Specimens of blood were withdrawn under oil from the jugular veins, care being taken to avoid stasis. The methods used in the analysis of blood, urine and diet specimens were the same as those described previously (3). After a suitable control period the animals were adrenalectomized.[4]

OBSERVATIONS

A daily subcutaneous injection of 1.5 to 3.0 mg. of desoxy-corticosterone acetate was sufficient to prevent the development of symptoms of adrenal insufficiency following bilateral adrenalectomy (dog 210, fig. 1). Throughout

[1] This study was aided by a grant from the Committee on Research in Endocrinology, National Research Council.

[2] The desoxy-corticosterone acetate (synthetic) used in this study was supplied by the Ciba Pharmaceutical Corp. through the courtesy of Doctors K. Meischer and E. Oppenheimer.

[3] The milk used in these studies was supplied through the courtesy of the Irradiated Evaporated Milk Institute.

[4] Under spinal anesthesia a single stage bilateral adrenalectomy was performed by Doctor Warfield M. Firor of the Department of Surgery, The Johns Hopkins University and Hospital. We wish to acknowledge our appreciation of his assistance and cooperation.

this study the animal was maintained on a diet of low sodium and chloride content. No additional sodium chloride therapy was given during the post-operative period. Preoperatively, during a 6-day period, the average daily renal excretion of sodium, chloride and potassium was found to be 16.5, 19.6 and 11.7 m.eq. respectively. Post-operatively during a period in which the animal received 1.5 to 2.0 mg. of desoxy-corticosterone acetate daily, the renal excretion of sodium, chloride and potassium was found to be 7.8, 11.1 and 11.2 m.eq. per day respectively. On the day preceding operation the animal's weight was 10.30 kg. On the 12th day after operation the animal

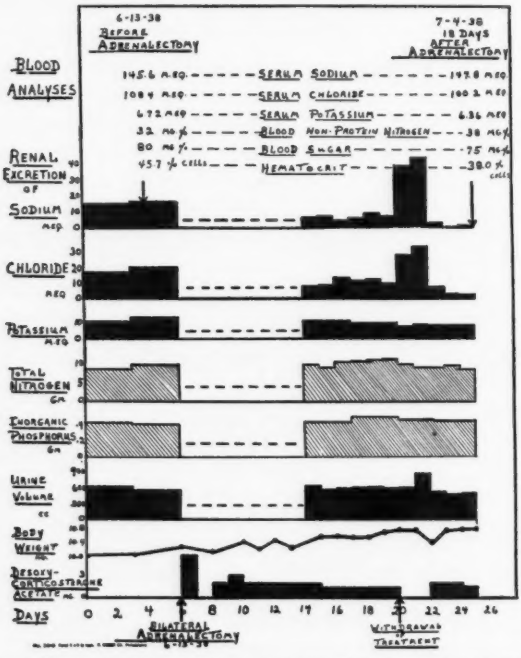

Fig. 1. Dog 210. THE EFFECTIVENESS OF A SINGLE DAILY INJECTION OF DESOXY-CORTICOSTERONE ACETATE IN MAINTAINING THE ELECTROLYTE BALANCE OF A BILATERALLY ADRENALECTOMIZED DOG. Throughout this study the animal was fed a diet which contained by analysis 14.1 m.eq. of Na, 12.3 m.eq. of Cl, 19.8 m.eq. of K, 0.550 gm. of total P, and 11.48 gm. of total N per day.

weighed 10.75 kg., a gain of 0.45 kg. On the 13th post-operative day withdrawal of desoxy-corticosterone acetate treatment resulted in weight loss (0.35 kg.), a diuresis associated with a markedly increased sodium and chloride excretion (42.8 and 31.6 m.eq. per day respectively), and a slightly reduced potassium excretion (9.3 m.eq. per day) The animal developed symptoms of adrenal insufficiency and 48 hours after treatment had been discontinued it was felt advisable to resume the injections of hormone. At this time desoxy-corticosterone acetate treatment (2 mg. injected once daily) resulted in marked improvement in the animal's condition, gain in body weight, and a markedly reduced excretion of sodium and chloride, the total daily excretion

being 2.7 and 5.2 m.eq. respectively. During the third to sixth days following the resumption of treatment (this period is not shown in fig. 1) the average daily quantity of potassium excreted in the urine was 16.9 m.eq. in contrast to 9.3 m.eq. excreted during the withdrawal period. Throughout this experiment no significant variations were noted in the renal excretion of inorganic phosphorus or total nitrogen.

Three days after resuming desoxy-corticosterone acetate treatment (18

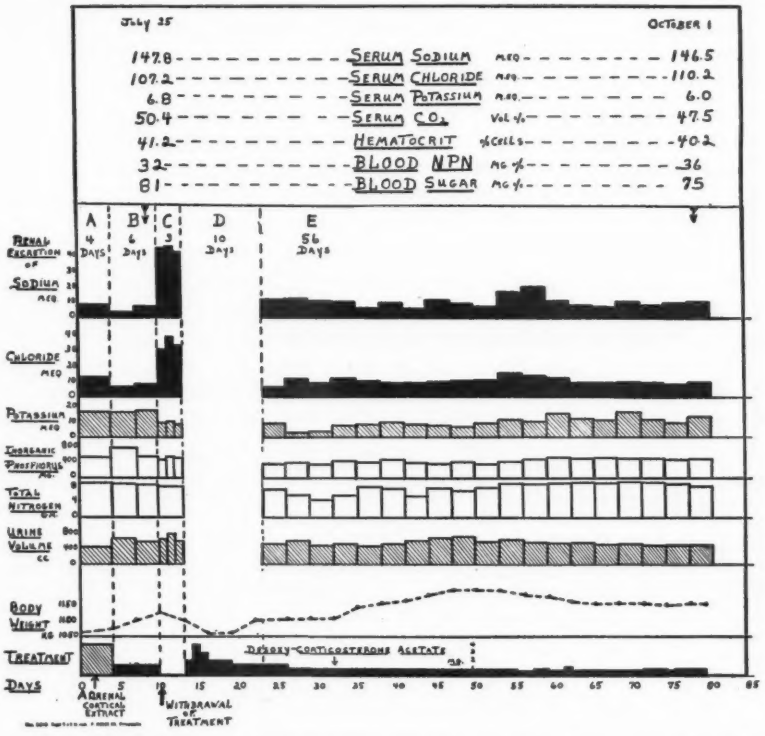

Fig. 2. Dog 211. The effectiveness of single daily injections of desoxy-corticosterone acetate in maintaining a bilaterally adrenalectomized dog. Throughout this study the animal was maintained on a diet of low Na and chloride content which contained by analysis 14.1 m.eq. of Na, 12.3 m.eq. of Cl, 19.8 m.eq. of K, .550 gm. of total P and 11.48 gm. of total N per day.

days following bilateral adrenalectomy) analysis of a specimen of blood showed values which approximated closely those observed prior to operation (fig. 1.).

Desoxy-corticosterone acetate (1.5 mg. injected once daily) was substituted successfully (fig. 2, dog 211) for injections of adrenal cortical extract[5] (the equivalent of 200 gm. of fresh cortex having been injected twice daily). Associated with desoxy-corticosterone acetate treatment there was a con-

[5] We are indebted to Doctor David Klein of the Wilson Laboratories, Chicago, Illinois, for the generous supply of adrenal cortical extract.

tinued gain in weight and a marked retention of sodium and chloride (section *B*, fig. 2). It appeared that a single daily injection of 1.5 mg. of desoxy-corticosterone acetate exceeded the daily requirement of hormone necessary for maintenance. Withdrawal of treatment (section *C*, fig. 2) resulted in a marked diuresis of sodium and chloride associated with potassium retention and weight loss. The excretion of sodium and chloride increased from an average daily value of 4.9 and 6.4 m.eq. respectively to 43.1 and 33.8 m.eq. respectively. During the same period potassium excretion decreased from a daily average of 16.4 m.eq. to 9.2 m.eq. Symptoms of severe adrenal insufficiency developed during the withdrawal period. It required approximately 10 days of treatment with desoxy-corticosterone acetate (1.5 to 4 mg. daily) to restore the animal to health. The tremendous loss of sodium (more than 100 m.eq. in 3 days), the limited sodium available in the diet (approximately 8 to 12 m.eq. per day) and the relatively small quantity of hormone used (approximately twice maintenance dose) contributed to the delayed recovery of the animal. Subsequently (period *E*, fig. 2) it was noted that a single daily injection of 1 to 1.2 mg. of desoxy-corticosterone acetate was sufficient to maintain the body weight of the animal. Reduction of this dose to 0.8 mg. per day resulted in weight loss and an appreciable increase in the renal excretion of sodium and chloride.

Seventy-three days after the institution of desoxy-corticosterone acetate therapy, analysis of the blood revealed essentially normal values (fig. 2). This animal has now been maintained for more than 150 days on a diet of low sodium and chloride content by means of desoxy-corticosterone acetate therapy. During this period withdrawal of desoxy-corticosterone acetate treatment has been attempted on three occasions. Following withdrawal of treatment there has occurred in each instance a diuresis associated with an increased excretion of sodium and chloride, potassium retention, weight loss and the development of signs of adrenal insufficiency. In each instance desoxy-corticosterone acetate treatment, without added sodium chloride, has corrected the altered electrolyte balance and has restored the animal to health.

Withdrawal of added sodium chloride from the diet of an adrenalectomized dog or a patient with Addison's disease, increases greatly the demand for adrenal cortical hormone and frequently precipitates a crisis. Such a procedure constitutes an additional method of testing the efficacy of any substance suspected of having 'cortin-like' properties. Two such experiments have been performed (fig. 3, 4). It will be noted (fig. 3, dog 209) that the substitution of desoxy-corticosterone (1.5 mg. daily) for adrenal cortical extract (3 cc. twice daily, representing a total daily dose of 240 gm. of fresh cortex) resulted in weight gain despite the rapid reduction of added sodium chloride (fig. 3). A positive sodium and chloride balance was attained 8 days after the completion of sodium chloride withdrawal. Analysis of the blood on the eleventh day after withdrawal of sodium chloride showed normal values (fig. 3). Withdrawal of desoxy-corticosterone acetate therapy at this time resulted in a marked diuresis, increased loss of sodium and chloride, potassium retention, loss of body weight, hemoconcentration and marked changes in

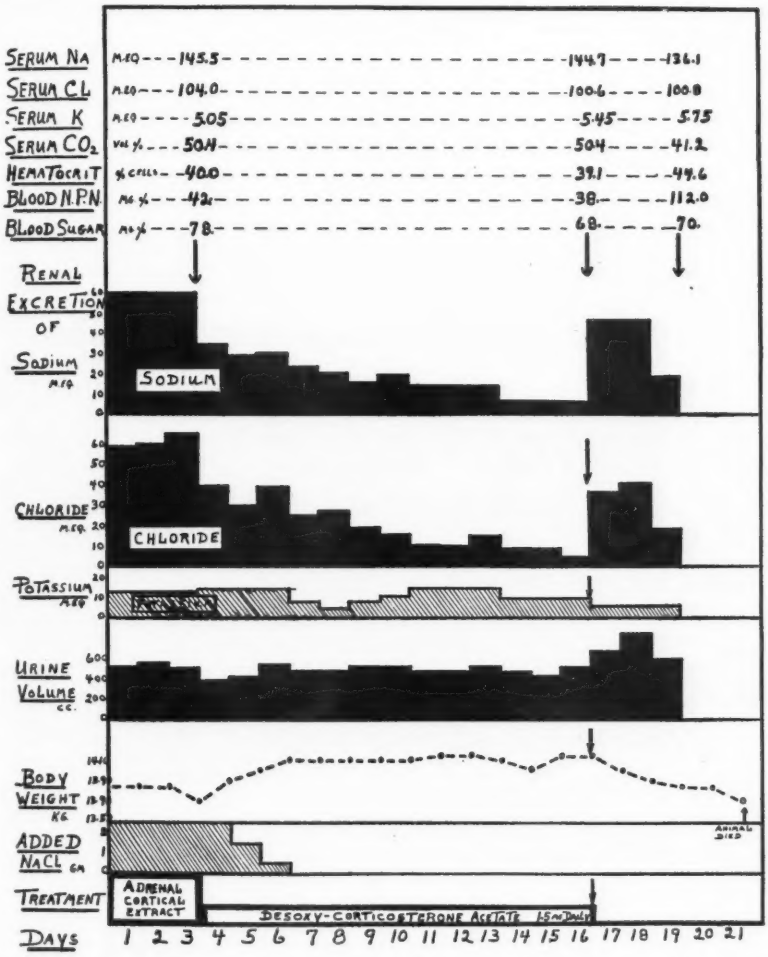

Fig. 3. Dog 209. THE EFFECTIVENESS OF A SINGLE DAILY INJECTION OF DESOXY-CORTICOSTERONE ACETATE IN MAINTAINING AN ADRENALECTOMIZED ANIMAL IN GOOD CONDITION FOLLOWING THE WITHDRAWAL OF BOTH ADRENAL CORTICAL EXTRACT AND NaCl THERAPY. From the 1st through the 4th days the total intake of Na was 56.9 m.eq. and chloride 55.1 m.eq., on the 5th day 39.8 m.eq. of Na and 38.0 m.eq. of chloride, on the 6th day 31.2 m.eq. of Na and 29.4 m.eq. of chloride; during the rest of the experiment 14.1 m.eq. of Na and 12.3 m.eq. of chloride, per day; the K intake remained constant throughout the study (19.8 m.eq. per day).

the values of the blood constituents. Death of the animal occurred 5 days after withdrawal of desoxy-corticosterone acetate treatment.

In a second experiment (fig. 4, dog 210) an attempt was made to reduce the duration of the negative sodium and chloride balance following the withdrawal of added sodium chloride by increasing the dosage of desoxy-corticosterone acetate injected during this period. In the previous experiment (fig. 3, dog 209) it required 8 days of treatment (a single daily injection of 1.5

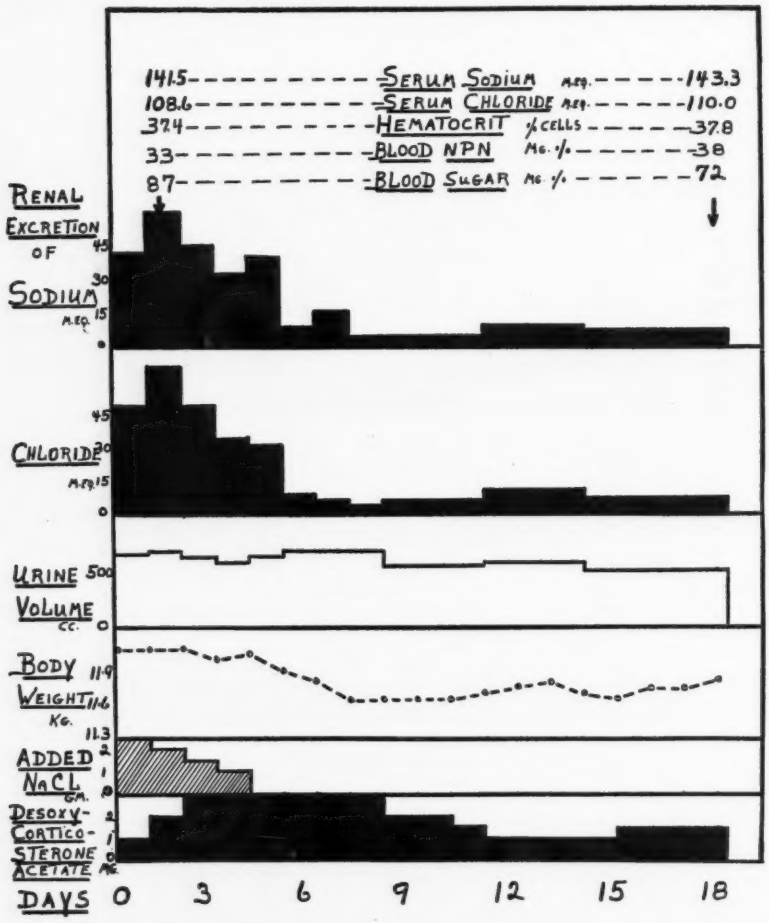

Fig. 4. Dog 210. The effectiveness of a single daily injection of desoxy-corticosterone acetate in maintaining an adrenalectomized animal following the withdrawal of NaCl. The intake of Na and chloride during this experiment was as follows.

	Na	Cl
1st day	56.9 m.eq.	55.1 m.eq.
2nd day	48.3 m.eq.	46.5 m.eq.
3rd day	39.8 m.eq.	38.0 m.eq.
4th day	31.2 m.eq.	29.4 m.eq.

5th and succeeding days sodium 14.1 m.eq., chloride 12.3 m.eq.

mg. of desoxy-corticosterone acetate) to restore a positive sodium and chloride balance following the withdrawal of added sodium chloride therapy. In the present experiment by increasing the daily injection of desoxy-corticosterone acetate to 3 mg. a positive sodium and chloride balance was established 38 hours after the withdrawal of added sodium chloride (fig. 4). Fifteen days after the withdrawal of added sodium chloride, blood analysis

showed essentially normal values. It appeared that on a diet of low sodium and chloride content the maintenance dose of desoxy-corticosterone acetate for this animal was 1.0 to 1.5 mg. per day. Subsequently the withdrawal of desoxy-corticosterone acetate treatment (not shown in fig. 4) resulted in a weight loss, a marked diuresis associated with an increased excretion of sodium and chloride, retention of potassium and the development of the signs and symptoms of adrenal insufficiency. Treatment with desoxy-corticosterone acetate at this time resulted in marked improvement in the animal's condition and in restoration of electrolyte balance.

In addition to the experiment reported in detail it has been found possible to maintain dogs *215* (9.00 kg.) and *217* (9.35 kg.) in good condition following a bilateral single stage adrenalectomy by means of 1 to 1.5 mg. of desoxy-corticosterone acetate injected once daily. During these studies the animals were fed a diet low in sodium and chloride.

DISCUSSION

A single daily injection of 1 to 1.5 mg. of desoxy-corticosterone acetate was sufficient to maintain an adrenalectomized dog (10 kg.) in good condition despite a diet low in sodium and chloride content and moderately high in potassium. The maximum survival period for an untreated dog on this diet was 4 to 5 days. A single injection of the hormone in oil appeared to be effective throughout the 24-hour period. It required approximately 3 to 4 cc. twice daily of injected adrenal cortical extract (this quantity representing a total daily dose of 240 to 320 gm. of fresh cortex) to maintain a 10-kg. dog on the diet used in these studies. It would therefore appear that 1.5 mg. of desoxy-corticosterone acetate injected once daily, was equal in effect to a daily dose of 6 to 8 cc. of adrenal cortical extract (the equivalent of 240 to 320 gm. of fresh cortex) injected subcutaneously in divided doses.

Withdrawal of desoxy-corticosterone acetate treatment resulted in a prompt diuresis accompanied by a marked increase in the excretion of soduim and chloride, a decrease in potassium excretion, hemoconcentration, a rise in blood non-protein nitrogen and the development of symptoms of adrenal insufficiency.

As previous studies (4) have indicated, in the adrenalectomized dog maintained under constant metabolic conditions, a change in the renal excretion of electrolytes provides the first detectable evidence of ensuing insufficiency.

Although the most striking changes observed in either the normal (3) or adrenalectomized dog injected with desoxy-corticosterone acetate were the retention of sodium and chloride and the increased excretion of potassium it is by no means certain that these are necessarily the only changes produced by desoxy-corticosterone acetate treatment. It does appear, however, that in adrenalectomized dogs there is a rapid return to health and activity following the restoration of electrolyte balance as the result of desoxy-corticosterone acetate treatment.

SUMMARY

Experiments on bilaterally adrenalectomized dogs fed a diet of low

sodium and chloride content indicate that the daily subcutaneous injection of 1 to 1.5 mg. of desoxy-corticosterone acetate (synthetic) dissolved in oil will maintain an animal weighing approximately 10 kg. Withdrawal of desoxy-corticosterone acetate treatment is followed by a prompt change in the renal excretion of electrolytes and the development of signs of adrenal insufficiency. Resumption of desoxy-corticosterone acetate treatment corrects the altered electrolyte balance and restores the animal to normal health and vigor.

We wish to acknowledge the technical assistance and cooperation of Kay Eisenberg and William Sause in this study.

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THE RATE OF INCREASE IN HYPOPHYSEAL GONADOTROPIC CONTENT FOLLOWING OVARIECTOMY IN THE RAT, WITH OBSERVATIONS ON GLAND WEIGHTS[1]

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IN 1929, Evans and Simpson (1) published a report on the rate of increase in pituitary gonadotropic potency following ovariectomy. Hypophyses from rats spayed previously were implanted into immature rats, and ovary weights were taken as the index of gonadotropic activity. The results indicated that a great increase occurred within two weeks after castration, and that the increase thereafter was at a slower rate. Since these authors did not measure hypophyseal potency in terms of some standard unit, and because the implantation methods are subject to considerable error, it was thought worthwhile to re-investigate the problem, using the improved technics of Heller, Lauson and Sevringhaus (2), and to extend it by observations on the effect of castration upon various gland weights.

METHODS

Healthy female albino rats of the Sprague-Dawley strain,[2] maintained on a liberal synthetic diet, were autopsied 5, 10, 20, 30, 60 and 120 days after ovariectomy. To permit direct comparison of organ weights, all groups were castrated at such age as would make them exactly 24 weeks old at autopsy. A group of normal females was also killed at this age. All rats were born within a period of 2½ months and were kept under a constant regime throughout. There were from 10 to 20 donor rats in each group.

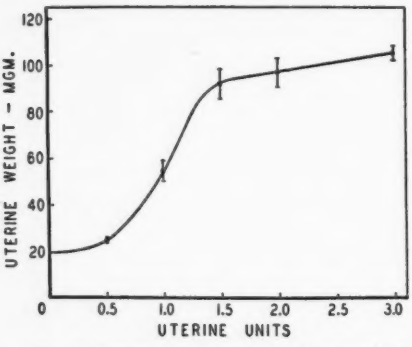
At autopsy, body weight and length were measured with the rats under deep ether anesthesia. After partial recovery from the anesthetic, they were killed by rapid decapitation and bled as completely as possible. Pituitary, thyroid, thymus, adrenals and kidneys were weighed. The pituitaries from all rats of each group were pooled, macerated and suspended in sterile distilled water as previously described (2). One-half cc. of the suspensions was injected twice daily for 3 days into 22-day-old female rats, weighing 35 to 40 gm. At autopsy on the morning of the 4th day, the uteri (minus fluid) and ovaries were weighed.

Sixty test rats were given total doses of 0.5, 1.0, 1.5, 2.0, and 3.0 mg. of fresh whole pituitary from the 20-day castrate group. The uterine weight curve obtained serves as a standard of reference for all other groups. This curve, with standard errors of the means, is shown in figure 1. The abscissa is graduated in terms of 'castrate uterine units,' an arbitrary unit representing the uterine weight produced by one mg. of 20-day castrate female rat pituitary. This unit differs from the 'minimal uterine unit' previously employed (2, 3). It is approximately equal to the unit described in our recent study of the gonadotropic content of the hypophysis throughout the life

[1] This research was supported in part by a special grant from the Wisconsin Alumni Research Foundation and by a research fund furnished by the Schering Corporation.
[2] We are grateful to Sprague-Dawley, Inc. for gratuitously furnishing the adult rats used in this investigation.

cycle of the normal female rat (4). The 'castrate' unit, however, has no other significance than to permit easy comparison among the groups of rats in this paper.

The pooled hypophyses from the 24-week-old normals and from the castrate groups were assayed on one to three groups of 12 test animals. Two or three dose levels of pituitary were used in all groups except the normal. A total of 107 donors and 271 test rats were employed. By referring the uterine weight response to the curve in figure 1, and knowing the average pituitary weight, the number of units of gonadotropic complex per average hypophysis was readily calculated for each group.



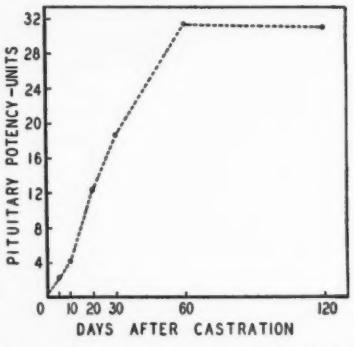


Fig. 1. Dosage-response curve based on results from the 20-day castrate pituitaries (table 1). One unit is equal to the activity of one mg. of whole fresh pituitary from female rats, ovariectomized 20 days prior to autopsy, when administered as described in the text. This curve formed the basis for estimating the gonadotropic content of the hypophyses of all other groups.

Fig. 2. Rate of increase in pituitary gonadotropic potency following ovariectomy. The points represent the calculated total number of uterine units contained in the average pituitary of each group (column 8, table 1).

RESULTS AND DISCUSSION

a), *Rate of increase in gonadotropic potency following ovariectomy.* Table 1 summarizes the results of the assays described above. The total doses of fresh whole pituitary administered in 6 injections over 3 days are given in the 3rd column. The mean uterine weights (column 4) were fitted to the curve, giving estimates of the number of uterine units per dose (column 5). From these estimates and the mean pituitary weight (column 6), the average number of units per gland was calculated (column 7). The several estimates for each group, based on the several doses, were averaged to give the results in the 8th column. The final column emphasizes the tremendous increase in potency resulting from gonadectomy.

The rate of increase is shown graphically in figure 2. There is more than a 3-fold increase by the 5th day, and by the 10th day the potency has been increased 7 times. The increase is most rapid between the 10th and 20th days, the pituitary content on the 20th day being three times as great as on the 10th day. By the 60th day the pituitary contains 52 times as much active hormone as does the normal. The curve given by Evans and Simpson, based on ovary weight response to implantation of 4 hypophyses, shows that the normal pituitary produces 16 mg. ovaries, and that one week after ovariectomy the recipient ovary response is 38 mg. At 2 weeks, 1 month, 2 months, and 6 to 8 weeks after castration, the values are 84, 92, 113.5 and 130.8 mg., respectively. Thus by two months after gonadectomy a 7-fold increase in

ovary weight actually represents approximately a 50-fold increase in gonadotropic content. The differences between our curve and that of Evans and Simpson are largely due to the fact that increase in ovary weight in response to increasing gonadotropic dosage is not a linear function.

TABLE 1. RESULTS OF GONADOTROPIC ASSAYS OF PITUITARIES FROM 24-WEEK-OLD FEMALE RATS OVARIECTOMIZED 0 TO 120 DAYS PREVIOUSLY

Donor group	No. of test rats	Total dose of pituitary mg.	Mean uterine weight ± S.E. mg.[1]	Castrate uterine units per dose	Mean donor pituitary weight mg.	Uterine units per average pituitary	Aver. no. of uter. units per aver. pit.	Potency increase over normal
Normals	12	19.8	45.1±3.6	0.9	13.2	0.6	0.6	—
5-day	12	5.6	44.7±4.2	0.9		1.8		
Castrate	12	11.2	102.5±7.3	2.6	11.2	2.6	2.2	3.7
	12	1.5	31.4±5.0	0.7		4.9		
10-day	12	4.0	77.8±5.5	1.2	11.8	3.7	4.2	7.0
Castrate	12	7.0	101.4±5.7	2.5		4.0		
	12	0.5	25.0±1.4	0.5		12.4		
	12	1.0	54.4±4.2	1.0		12.4		
20-day	12	1.5	92.1±6.6	1.5	12.4	12.4	12.4	20.7
Castrate	12	2.0	96.9±6.0	2.0		12.4		
	12	3.0	105.5±3.2	3.0		12.4		
30-day	12	0.5	36.5±3.4	0.8		21.7		
Castrate	12	1.0	62.7±2.9	1.1	14.3	15.7	18.7	31.2
	12	0.25	28.2±2.2	0.6		33.3		
60-day	12	0.75	93.5±5.8	1.6	13.9	29.6	31.4	52.7
Castrate	12	1.5	117.4±7.7	>3.0		>27.8		
120-day	12	0.25	23.3±0.8	0.4		30.4		
Castrate	12	0.5	47.8±3.0	0.9	17.3	31.8	31.1	51.9
Untreated Control Test Rats	55	—	19.6±0.3	—	—	—	—	—

[1] S. E. equals standard error of the mean $= \frac{\sqrt{\frac{\Sigma x^2}{N-1}}}{\sqrt{N}}$

It may be mentioned that ovary weights in nearly all assay groups were essentially unchanged from control level. This is in accord with the finding of Heller, Lauson and Sevringhaus (2) that the uterine end-point is about six or eight times as sensitive as the ovarian weight criterion, when castrated female rat pituitaries are the source of gonadotropic complex.

Comparison of figure 1 with the curve reported by the above authors (2), reveals certain differences dependent upon the fact that nine injections were given over a 4½ day period in the earlier work. The longer period resulted in lower maximum uterine weight, somewhat greater variation on each dose, and a higher threshold for minimal stimulation. A study was recently made in this laboratory in which the 3 and the 4½ day periods were compared, using the same batch of pituitary suspension (from 20-day castrate female rats) for both groups. The longer period gave results agreeing closely with the earlier work (2), while the results of the 3-day period were in harmony with

those shown in figure 1. Thus, the potency of 20-day castrate pituitaries has been essentially identical on three different occasions, indicating that the gonadotropic content of this type of pituitary is remarkably constant.

b), *Body weight and length.* Table 2 summarizes these data, as well as those of the gland weights. Both weight and length increase with time after gonadectomy, indicating true growth. This is in agreement with the findings

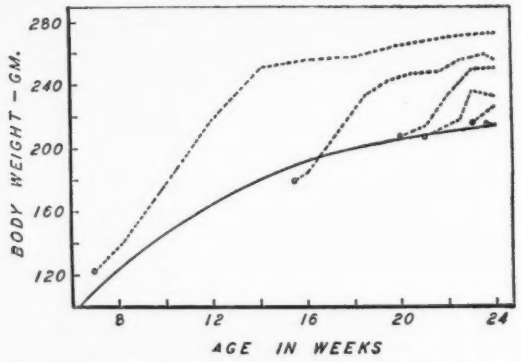


Fig. 3. Growth curves of female rats castrated 120, 60, 30, 20, 10 and 5 days prior to autopsy at the age of 24 weeks (broken lines) compared to the growth of normal females of the colony (solid line). The small circles indicate age and weight at time of ovariectomy.

of Holt, Keeton and Vennesland (5) and contrary to those of Freudenberger and Billeter (6) and Billeter (7), who found no increase in body length. Weight curves of the castrated groups are compared to the normal curve for our colony in figure 3.

c), *Pituitary and thyroid weights.* These glands failed to show any consistent trend. However, both were heaviest in the largest animals, namely, the 120-day group. There is no evidence of atrophy, since no group decreased significantly below the normals, and only the 120-day castrates show significant enlargement.

d), *Thymus weight.* The rapidity with which this organ hypertrophies is strikingly shown in table 2. By the 10th day after castration, this weight is increased nearly 60%, in 20 days the increase is 220% above normal, and by the 30th day a maximum increase of 240% is attained. The average weight in this group (416 mg.) considerably exceeds the maximum weight of the thymus in normal females of our colony (306 mg. at the time of puberty). We have no explanation for the decrease found in the rats autopsied 5 days after ovariectomy, except that it may possibly be related to the shock of the operation (8).

e), *Adrenal weight.* The adrenals of the 30, 60 and 120-day groups do not differ significantly from the normal, suggesting that there was no atrophy following castration. Whereas body growth was accelerated by gonadectomy the adrenal growth seems to have continued at the same rate as in the normal rats. This is contrary to the observations of Freudenberger and Billeter (6), Billeter (7), Blumenfeld (9), Andersen and Kennedy (10) and Winter and Emery (11), all of whom found adrenal atrophy. In most of these cases, how-

ever, the period elapsing between spaying and autopsy was considerably longer than in our experiment. Our results are in agreement with those of Lauson, Heller and Sevringhaus (12), who found no adrenal atrophy 20 days after ovariectomy.

TABLE 2. SUMMARY OF BODY LENGTH AND BODY AND ORGAN WEIGHTS OF 24-WEEK-OLD FEMALE RATS CASTRATED 0 TO 120 DAYS BEFORE AUTOPSY. (MEAN ± STANDARD ERROR)[1]

Group	Normal	5 days after castration	10 days after castration	20 days after castration	30 days after castration	60 days after castration	120 days after castration
Body weight at castration —gm.	—	216±1.8	217±2.5	208±1.2	208±2.7	180±1.4	123±1.8
Body weight at autopsy —gm.	216±2.9	214±2.0	230±3.1	230±3.3	251±5.6	256±5.3	274±5.7
Body length at castration —cm.	—	20.6±0.1	20.7±0.1	20.3±0.1	20.2±0.1	19.5±0.1	16.8±0.1
Body length at autopsy —cm.	20.6±0.1	20.6±0.1	20.8±0.1	20.8±0.1	21.1±0.2	21.3±0.1	21.7±0.1
Pituitary weight—mg.	13.2±0.5	11.2±0.3	11.8±0.3	12.4±0.3	14.3±0.5	13.9±0.4	17.3±0.5
Thyroid weight—mg.	10.6±0.5	10.3±0.4	11.8±0.4	9.7±0.6	11.3±0.3	10.7±0.4	13.7±0.7
Thymus weight—mg.	173±8.8	144±10.1	273±15.9	379±18.3	416±30.9	362±21.7	311±22.7
Adrenal weight—mg.	51.1±1.6	59.1±1.9	55.9±1.1	57.4±2.2	52.2±1.3	50.3±1.2	48.9±1.8
Kidney weight—gm.	1.42±0.04	1.50±0.02	1.56±0.03	1.63±0.03	1.63±0.04	1.57±0.03	1.56±0.03
No. of rats per group	20	20	15	15	14	13	10

[1] Calculated from same formula as in table 1.

f), *Kidney weight.* The increase in kidney weight is roughly proportional to body growth following castration, except in the 60 and 120-day groups, in which kidney growth fails to keep pace with body growth. There is no significant difference among the kidney weights of the 10, 20, 30, 60 and 120-day groups.

SUMMARY

The rate of increase in pituitary gonadotropic potency following ovariectomy in the rat has been investigated. The gonad-stimulating content of the hypophyses from normal 24-week-old females and females of the same age gonadectomized 5, 10, 20, 30, 60 and 120 days prior to autopsy was evaluated in terms of an arbitrary uterine unit. The potency per gland in the respective castrated groups was found to be 3.7, 7.0, 20.7, 31.2, 52.7 and 51.9 times greater than normal.

Body weight and length increased in proportion to the time elapsed between castration and autopsy. There was no consistent effect on pituitary and thyroid weights, and no significant atrophy of the adrenals was found. Hypertrophy of the thymus reached a maximum increase of 240% over normal by the 30th day after ovariectomy. Increase in kidney weight was roughly proportional to body growth except in the 60 and 120-day castrates.

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THE INFLUENCE OF THE ANTERIOR PITUITARY GLAND ON PROTEIN METABOLISM[1]

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It is now well established that the anterior pituitary gland exerts a profound influence on protein metabolism (1). This may be exemplified by the fact that extracts of this gland when administered to normal animals (dog and rat), produce a diminution of nitrogen excretion (2–5), a decrease in the concentration of blood non-protein-nitrogen (3, 6) and a consistent decrease in the amino acids of liver, muscle and other tissues (7, 8). Such evidence can be interpreted as showing that under the influence of the pituitary gland there occurs either a retention of nitrogen and synthesis of protein, or a decreased catabolism of protein.

In a recent report (9) we have demonstrated that insulin plays an important rôle in protein anabolism in that a 'nitrogen-sparing effect' can be produced in normal, eviscerated and depancreatized animals. When this fact is correlated with the observations of Anselmino and Hoffman (10) and of Richardson and Young (11) that extracts of the anterior pituitary gland may be pancreatropic, the question arises as to whether the nitrogen-sparing effect of anterior pituitary extracts is due to a direct action on the muscles, or to some indirect effect via the pancreas. In order to obtain some information as to the influence of anterior pituitary extracts on the protein metabolism of the muscles, we studied the effect of such extracts in normal, eviscerated and depancreatized dogs. For, if these extracts always exert a "nitrogen-sparing action,' then similar results should be obtained in all three preparations. On the other hand, if the pancreas plays a rôle in the nitrogen-sparing action of the extracts, then it follows that the results should be different.

METHODS

The procedure employed for gauging the rate of protein metabolism, that is, for measuring the difference in the rate of anabolism and catabolism, consisted in determining the rate of non-protein-nitrogen and of amino acid nitrogen accumulation in the blood after bilateral nephrectomy (9).

One group of dogs was fasted for 96 hours before the onset of the experimental period and then subjected to a bilateral nephrectomy under sodium amytal anesthesia. A second group of dogs was depancreatized 96 hours before the experimental period, maintained without food or insulin, and then subjected to bilateral nephrectomy.

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The third group of 96-hour fasted dogs was completely eviscerated by a modification of the method of hepatectomy described by Markowitz, Yater and Burrows (13) and the blood sugar subsequently maintained at a normal level by the constant intravenous injection of a 30% solution of glucose at the rate of ⅛ gm. per kg. of body weight per hour. In the animals of all three groups, anesthesia was maintained throughout the period of study.

The various sets of animals were divided into control and anterior pituitary extract (APE) treated groups. The APE, consisting of a neutralized alkaline extract of beef anterior pituitary glands, was administered subcutaneously in a massive dose to all treated animals 12 hours before the onset of the experiment. The dose employed was equivalent to 2 gm. of fresh anterior lobe per kg. of body weight.

Blood samples for the determination of non-protein-nitrogen by a micro-Kjeldahl method and amino acid nitrogen by Danielson's method (14) were drawn from an exposed femoral artery at various intervals throughout the experiment.

RESULTS

The data are summarized in table 1 and figure 1. It is obvious from *A* & *B* (table 1) that the administration of APE results in a slight decrease in the rate of nitrogen accumulation in the blood, which signifies an increase in nitrogen retention by the tissues. This retention (18%) is of the same order as that observed by others (4) who employed the rate of urinary nitrogen excretion as the index of APE activity.

TABLE 1. THE CHANGE IN THE BLOOD NON-PROTEIN AND THE AMINO-ACID NITROGEN AFTER BILATERAL NEPHRECTOMY UNDER VARIOUS EXPERIMENTAL CONDITIONS

Experimental procedure	Blood N.P.N. after nephrectomy				Amino acids after nephrectomy		
	No. of expts.	Initial	12 hrs.	Rise in 12 hrs.	Initial	12 hrs.	Rise in 12 hrs.
A Normal	8	29.3	65.8	36.5	6.9	6.6	
B APE—Normal	5	25.7	55.7	30.0	6.6	6.8	0.2
C Depancreatized—Controls	8	40.6	100.3	59.7	10.2	11.6	0.6
D APE—Depancreatized	5	51.1	141.1	90.0			
E Eviscerated—Controls	10	32.0	52.7	20.7	8.1	22.7	14.6
F APE—Eviscerated	5	29.6	62.9	33.3	7.8	32.4	24.6

That the rate of protein breakdown is accelerated in the completely depancreatized dog, is clearly evidenced by *C* (table 1), where an increase of approximately 61% in the non-protein-nitrogen accumulation is obtained in the blood. When APE is administered to such animals, the rate of non-protein-nitrogen accumulation in the blood is still further accelerated (table 1, *D*), thus indicating that the rate of protein catabolism has been increased in consequence of the injection of APE, an effect which is opposite to that obtained in the normal dog. This is in accord with the observation that the administration of APE results in an increase in nitrogen and sugar excretion in the urine of depancreatized animals.

The data obtained with eviscerated dogs are summarized in table 1, *E* and *F*. From these it is obvious that the evisceration results in a diminution in the rate of protein catabolism and a reversal of the APE effect. Instead

of producing a decrease in the rate of non-protein-nitrogen accumulation, a marked increase in the rate of protein catabolism is indicated. That this is due to some effect on the muscle proteins is suggested by the fact that the amino acid nitrogen enters the blood of the APE treated eviscerated animal at a much greater rate than in that of the eviscerated control. Since the eviscerated animal is essentially a muscle preparation, the only source of these amino acids is the muscles. In figure 1, the data are so expressed that the effects consequent to APE administration can be noted by comparing

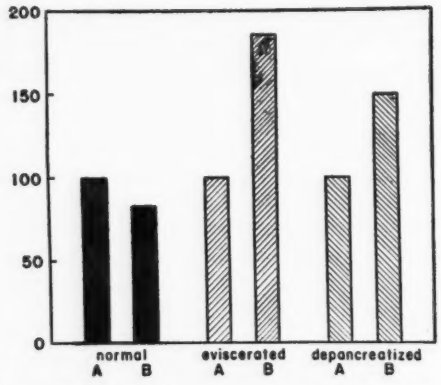

Fig. 1. The influence of APE administration on protein metabolism of normal, eviscerated and depancreatized dogs. The standard for comparison in each group is the rate of nitrogen accumulation in the blood of the controls (*A*) after bilateral nephrectomy. This rate is expressed as 100% with which are compared the APE treated animals (*B*).

with the non-treated controls of the particular group. Thus the controls for each group are expressed as 100%, and serve as the standard for the treated animals.

DISCUSSION

It is evident from our data that the administration of a crude neutralized alkaline extract of the anterior pituitary gland results in a retention of nitrogen in the tissues of the normal animal. This retention is the result of either an increase in protein synthesis or a decrease in protein breakdown. However, when the abdominal viscera are removed, so that the effect on the protein metabolism of the muscles now becomes apparent, the influence of the APE is in the opposite direction. Under these conditions an increase of approximately 80% in the rate of protein breakdown is observed. This suggests that the viscera either have an inhibitory effect on the action of the extract, or that while protein is undergoing hydrolysis in the muscle, some factor is producing a simultaneous anabolic effect.

The fact that in the absence of the pancreas, the over-all effect of APE administration is an increase in the breakdown of protein instead of a decrease, that is the depancreatized animal receiving APE behaves like the eviscerated animal receiving APE suggests that the presence of the pancreas

may be a factor in preventing the breakdown of protein, and may actually play a rôle in the synthesis of protein. That the pancreas probably can have such a function is indicated by the observation that insulin exerts a definite nitrogen-sparing action in normal, eviscerated and depancreatized dogs (9).

The findings of Anselmino and Hoffman, of Richardson and Young and of others, that the anterior pituitary may exert a pancreatropic effect, points to the possibility that the administration of an extract of this gland may stimulate the production of insulin. Furthermore, the studies of Richardson and Young (15) and Campbell and Best (16), which demonstrate that the permanent diabetes consequent to the continued administration of anterior pituitary extracts to dogs is associated with hydropic degeneration of the islands of Langerhans, are in accord with such a view if it can be assumed that the degenerative changes observed in those dogs are due to pancreatic exhaustion. That the latter is a possibility is suggested by the fact that the insulin content of the pancreas of a dog rendered diabetic by the administration of an anterior pituitary extract is very low (16).

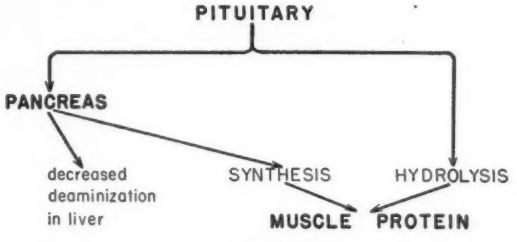

Fig. 2

On the basis of these considerations, it is possible to postulate that APE directly stimulates the hydrolysis of muscle protein, while at the same time it induces the pancreas to secrete insulin (fig. 2). Since the latter can increase the retention of nitrogen in the muscles, as well as decrease the oxidative breakdown of amino acids in the liver, the net result of the administration of an anterior pituitary extract to a normal animal will be a decrease in the rate of protein catabolism. Accordingly, the removal of the pancreas will result in an increase in the breakdown of muscle protein, while the removal of the pituitary may induce the opposite effect. From such an hypothesis, it can be anticipated that a permanent increase in the rate of protein breakdown (as occurs in permanent diabetes mellitus) will occur only if the secretion of insulin by the pancreas is inhibited or decreased.

SUMMARY

The administration of a crude alkaline extract of the anterior pituitary results in a decrease in the protein breakdown of normal dogs, an increase in the rate of protein catabolism in depancreatized dogs, and a similar increase in eviscerated dogs. It is suggested that the anterior pituitary gland exerts a

direct effect on the muscles, which is in a direction opposite to that observed in the intact animal. It is postulated that the synthesis of muscle protein consequent to the administration of extracts of the anterior pituitary may be dependent upon the simultaneous stimulation of the pancreas.

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CHEMICAL FRACTIONATION OF THE GONADOTROPIC FACTORS PRESENT IN SHEEP PITUITARY[1]

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STUDY OF THE FUNCTIONAL interrelationship of the pituitary with the gonads has yielded a number of facts of great importance, but many features of the mechanism of this reciprocal relationship are still obscure. Some of the results secured are actually contradictory to others and the situation in general has permitted undesirable freedom in interpretation. Conceptions as to this interrelationship have also doubtless been complicated by the postulation of several different gonadotropic principles (1, 2, 3). A prime reason for failure in arriving at a clear understanding of how many specific gonadotropic principles are actually secreted by the pituitary is the difficulty of adequately purifying pituitary fractions so that they will have a specific effect on the gonads.

In our preliminary studies of the gonadotropic principles in the sheep pituitary, it became apparent that several different biological effects—synergism, luteinization, and antagonism—were all given by the interstitial cell stimulating fraction when given in combination with the follicle stimulating fraction. It could, therefore, be justifiably assumed, as a working hypothesis, that the fraction repairing the interstitial tissue may in fact be identical with the luteinizing and antagonist factors. On the basis of these observations we directed our efforts to the chemical separation and purification of the follicle stimulating hormone (FSH) and the interstitial cell stimulating hormone (ICSH).

In this paper we wish to report on the chemical procedure employed and to comment briefly on certain biological properties of the two fractions as tested in the hypophysectomized immature female rat. A more detailed description of their physiological behavior will be published elsewhere.

Definition of units and method of standardization of FSH and ICSH. For the quantitative biological evaluation of these two factors we adopted the following definition of units.

FSH. One rat unit represents the minimal total amount one third of which, injected subcutaneously once daily for 3 days into hypophysectomized rats (26 to 28

[1] This investigation was aided by grants from the Boar i of Research of the University of California and the Rockefeller Foundation of New York City.

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days old at operation, 6 to 8 days post-operative interval before injection), causes resumption of follicular growth,[4] as evidenced at autopsy, 72 hours after onset of injections.

ICSH. One rat unit represents the minimal total amount one third of which, injected intraperitoneally once daily for 3 days into hypophysectomized rats (26 to 28 days old at operation, 6 to 8 days post-operative interval before injection), causes repair of the deficient interstitial tissue[5] of the ovary, as evidenced at autopsy, 72 hours after onset of injections.

We feel that crucial evidence for the purity of gonadotropic preparations must be sought in hypophysectomized animals since in the normal animal the secretion of the pituitary will no doubt interfere with the qualitative and quantitative response to the preparation injected. For physiological testing the preparations were generally dissolved in basic phosphate at pH 7.4.

EXPERIMENTAL

Extraction of FSH and ICSH. Since we found that in the extraction of the gonadotropic principles from the pituitary, such solvents as dilute sodium hydroxide, ammonium hydroxide, and pyridine would extract, at the same time, a comparatively large amount of other protein material which made further fractionation and purification more difficult, we chose 40% ethyl alcohol as an extractant.

Three hundred grams of acetone-dried whole sheep pituitary powder were extracted with 4 liters of 40% alcohol over a period of 2 days with continuous agitation, and a second extraction carried out similarly using 2 liters of the alcohol. The supernatant liquids were combined and filtered through coarse folded filter paper. The active material in the filtrate was precipitated by increasing the concentration of the alcohol to 80 or 85%. For maximum flocculation, the pH was adjusted to 5.5 by the addition of glacial acetic acid. After standing 3 to 5 days at room temperature, the precipitate was removed by centrifuging and dried with absolute ethyl alcohol and ether. The yield was usually somewhere between 12 and 15 gm.

It has been found that under the conditions outlined, practically all of the FSH and approximately 90% of the ICSH is extracted. As can be seen from table 1 the potency of the extract has been increased from 400 R.U. per gm. to 4000 R.U. per gm. in the case of the FSH principle, and from 1000 R.U. per gm. to 20,000 R.U. per gm. for the ICSH principle. This 40% alcohol powder has been found to contain about 10 to 20% inorganic material, the composition of which has not yet been determined.

Fractionation of the 40% alcohol powder. Forty gm. of the 40% alcohol preparation were extracted 3 times with dilute acetic acid at pH 4, 600 cc. of acid being used for the first extraction, 400 cc. for the second, and 200 cc. for the third. Each extraction was carried out at room temperature for 24 hours either with thorough stirring or shaking.[6]

[4] By 'follicular growth' is meant the occurrence of healthy (non-atretic) follicles with small antra.

[5] By 'repair of the interstitial tissue' is meant the resumption of a normal nuclear picture and abolition of the wheel pattern of the nuclear chromatin which has given these cells the designation 'deficiency cells.'

[6] All extractions were carried out in the same manner, thymol being used as a preservative to prevent proteolytic decomposition.

The acid-insoluble material, which is fairly high in amount (approximately 20 gm.), was found to be practically devoid of any FSH activity, but still retained some of the ICSH activity. The combined extracts were adjusted to pH 5 by the addition of 5N sodium hydroxide. A small precipitate which occasionally formed at this stage was removed by centrifugation and discarded since it was found to be free of gonadotropic activity. To the supernatant liquid an adequate amount of ammonium sulfate was added in order to saturate the solution to the extent of 20% with regard to the ammonium sulfate content (0.2 SAS).[7] If a precipitate was formed it was always comparatively small in amount (0.1 to 0.2 gm.) and was removed by centrifugation. The supernatant liquid was brought to 0.5 SAS. The precipitate formed at this stage represents the ICSH fraction (preparation *A*) which, however, is still contaminated with FSH and is further fractionated as described below under *A*. The supernatant liquid of the 0.5 SAS precipitate was brought to 0.8 SAS. The resulting precipitate represents the FSH fraction (preparation *B*) which is still contaminated with ICSH and is further purified as described under *B*.

Preparation A (ICSH fraction). The 0.5 SAS precipitate was treated with 200 cc. of dilute acetic acid at pH 4, in which it was generally found to be quite soluble. The solution was adjusted to pH 5 and then brought to 0.2 SAS, only a small precipitate being formed. After separation by centrifugation, the supernatant liquid was brought to 0.5 SAS. The second 0.5 SAS precipitate formed was again dissolved in 100 cc. of dilute acetic acid at pH 4, after which the solution was brought to 0.1 SAS by the addition of ammonium sulfate. A small precipitate resulted and was removed. The supernatant liquid was brought to a concentration of 0.4 SAS and the resulting precipitate extracted twice with 50 cc. of a 0.4 SAS solution at pH 4. Each extraction was carried out for 12 hours. The insoluble material was suspended in water and dialyzed.[8]

It has generally been found that some water-insoluble material of high ICSH potency precipitates out during dialysis. This precipitate was brought into solution by the addition of sodium hydroxide. After removal of any insoluble material a sufficient amount of alcohol was added to the solution to make the final alcohol concentration 80 to 85%. The solution was adjusted to pH 5 by the addition of acetic acid. The precipitate formed, representing the main ICSH fraction, was removed by centrifuging and then dried with absolute alcohol and ether. The yield was approximately 1 gm.

Preparation B (FSH fraction). The first 0.8 SAS precipitate was extracted with 400 cc. of dilute acetic acid at pH 4, in which it should be readily soluble. After adjusting to pH 5 the solution was brought to 0.2 SAS, at which concentration a small precipitate often formed which was removed. The solution was next brought to 0.5 SAS and the resulting precipitate

[7] SAS will be used throughout this paper as an abbreviation for saturated ammonium sulfate solution, e.g., 0.2 SAS means a solution 20% saturated with respect to ammonium sulfate. In the fractional ammonium sulfate separation, we adopted the following procedure: the required amount of solid ammonium sulfate was gradually added with stirring, which was continued for 4 hours, and the solution then allowed to stand for 24 hours.

[8] Dialysis of the various fractions was carried out against distilled water at 0° C. for 3 days, with three daily changes of the dialysate water.

removed by centrifugation. The supernatant liquid from this precipitate was brought to 0.8 SAS and the precipitate formed extracted twice with 50 cc. of a 0.4 SAS solution at pH 4. The insoluble part was removed and the supernatant liquid again brought to 0.8 SAS. The resulting precipitate was centrifuged, suspended in water and dialyzed. Very little, if any, insoluble material appeared during dialysis and in such instances was removed by centrifugation. Alcohol was added to the solution to a concentration of 80 to 85% and the pH adjusted to 5. After standing for 2 days, the precipitate was centrifuged and dried with absolute alcohol and ether. The yield of this main FSH fraction was approximately 1 gm. It was found to be quite soluble in water.

TABLE 1. POTENCY OF GONADOTROPIC PREPARATIONS FROM SHEEP PITUITARY.
(As Tested in Hypophysectomized Immature Female Rats)

Material	FSH Subcutaneously		ICSH intraperitoneally	
	Min. Dose *mg.*	R.U./gm.	Min. Dose *mg.*	R.U./gm.
Dried glands	2.5	400	1.0	1,000
40% Alcohol extract	0.25	4,000	0.05	20,000
Main fractions	0.025	40,000	0.0125	80,000
Approx. purification	1:100		1:80	

As can be seen from table 1 the purification process increased the potency (in terms of R.U. per gm.) 100 times for the FSH fraction and 80 times for the ICSH fraction, as compared to the activity of the dried glands. The actual yield represented by the main fractions is admittedly comparatively low since it represents only the recovery of about 10% of the original physiological activity. One has to consider, of course, that a large amount of the original activity is present in the various intermediate fractions obtained in the separation of these two principles. These fractions can be combined and worked up again according to the procedure just outlined.

Biological Behavior of the Follicle Stimulating Fraction

The FSH fraction, when prepared as outlined, has been found to show the following biological properties. The M.E.D. causing resumption of follicular growth in hypophysectomized rats is about 0.025 mg., the same effect being obtained whether subcutaneous or intraperitoneal injections were made. In addition, the purity of the FSH preparation can be judged by determining the multiples of the M.E.D. which can be given before the influence of biologically active contaminants (in this case mainly the ICSH factor) can be detected. It was found that generally 10 to 20 times the minimal dose of this purified FSH fraction could be given subcutaneously to hypophysectomized animals before repair of the deficient interstitial tissue or formation of corpora lutea could be observed. We are of the opinion that the luteinization obtained with high doses of the purified FSH is more of the granulosa type in contrast to the thecal luteinization which is produced by, *a*), combination of low doses of this FSH with either ICSH or prolan, *b*), insufficiently fractionated gonadotropic pituitary preparations, and, *c*),

administration of pregnant mare serum. The question whether the luteinization resulting from high doses of FSH is due to the factor itself or because of contamination with ICSH cannot be answered at present. A definite solution of this problem may perhaps be reached only if one succeeds in obtaining the FSH principle in crystalline form.

Biological Behavior of the Interstitial Cell Stimulating Fraction

The following physiological properties have been found for the ICSH fraction when prepared according to the procedure outlined. The M.E.D. capable of repairing the deficient interstitial tissue in the ovary of the hypophysectomized rats, when given intraperitoneally, has generally been found to be about 0.0125 mg. Using the method of subcutaneous injection, the M.E.D. was found to be higher, the ratio being in the vicinity of 1 to 4. It has been found that approximately 10 to 20 times the M.E.D. can be given subcutaneously to hypophysectomized animals before the development of follicles and formation of corpora lutea can be observed. When injected subcutaneously into normal immature female rats small doses (1 to 4 R.U.) show no striking physiological response, while higher doses (25 to 50 R.U.) will produce an increase in ovary weight and formation of corpora lutea.

Combination Effects of FSH and ICSH

When the ICSH preparation is combined with the FSH factor at certain dose levels and given subcutaneously to hypophysectomized rats, synergism is observed in terms of increased follicular development and increased ovary weight with simultaneous repair of the deficient interstitial tissue. At higher levels of ICSH luteinization occurs. When the two factors are given intraperitoneally, however, to hypophysectomized rats, an antagonistic effect is produced, as manifested by an inhibition of follicular development. Furthermore, it has been found that the ICSH fraction has an antagonistic effect on pregnant mare serum[9] when both are injected intraperitoneally into normal immature rats. This antagonism was indicated by reduction of ovarian weight.

DISCUSSION

The chemical procedure reported makes possible the separation of two gonadotropic factors from dried sheep pituitary, namely a follicle stimulating factor (FSH) and an interstitial cell stimulating factor (ICSH). It is obvious that our two gonadotropic fractions are still contaminated with each other. Further chemical purification is unquestionably desirable. We are aware of the possibility that one could perhaps work out a shorter and more convenient chemical method for the separation of these two gonadotropic principles. However, our main object in the investigation recorded herein was to fractionate the gonadotropic physiological activity of sheep pituitary powder.

From the observed biological properties of the two gonadotropic fractions, FSH and ICSH, briefly recorded in this paper, it appears that the

[9] Preliminary experiments on the chemical fractionation of the gonadotropic hormones present in pregnant mare serum have indicated the presence of two gonadotropic factors, namely FSH and ICSH.

development of the ovary (as studied in the rat) depends on the balanced physiological effect of FSH and ICSH.[10] We were able to demonstrate, *a*), synergism (activation), *b*), luteinization and *c*), antagonism, by the combined administration of certain adequate doses of these two factors under given modes of injection. The assumption of a separate synergist or activator apart from the FSH and of a separate antagonist and luteinizing hormone apart from the ICSH (2, 3) appears to be at present unjustified.

Comparison with results of similar nature reported from other laboratories (1, 4) is rather difficult, since different methods of standardization have been employed and often dosages have been given as gram-equivalent of pituitary powder and not in biological units. Fevold, who in collaboration with Hisaw and Greep, has done extensive work on the relationship of the pituitary to the gonads, has recently published a modified procedure for separating the follicle stimulating hormone and the luteinizing hormone of the pituitary (4). We found that the FSH factor prepared according to this procedure is about one-third as active as our purified FSH preparation. The LH factor is much less potent than our purified principle (ICSH) in terms of the M.E.D. capable of repairing the deficient interstitial tissue in hypophysectomized immature female rats. The discrepancy in the physiological potency of our fraction (ICSH) and that of Fevold (LH) can perhaps be explained by the fact that Fevold precipitates the luteinizing factor at pH 4 while we extract the same principle with dilute acetic acid at pH 4. As can be seen from our procedure, a comparatively large amount of inert protein (at least with respect to gonadotropic activity) is insoluble at that pH. Since we observed that the ICSH (LH) is quite readily adsorbed on protein material, we believe that this factor prepared according to the method of Fevold is still greatly contaminated with other protein material.

SUMMARY

A method has been described for the separation of two gonadotropic fractions from sheep pituitary, *a*) FSH, M.E.D. 0.025 mg. in hypophysectomized female rats, using subcutaneous and intraperitoneal methods of injection, and *b*) ICSH, M.E.D. 0.0125 mg. in hypophysectomized animals upon intraperitoneal injection. Either fraction is free of the other up to 10 to 20 times the M.E.D. The separation and purification of the two fractions described has increased the potency about a hundredfold over that characterizing the initial dried sheep pituitary powder.

A brief description of the physiological behavior of each fraction, and of the combination of the two, has been given, showing that such physiological effects as synergism (activation), luteinization and antagonism can be secured by simultaneous administration of the two fractions designated as FSH and ICSH.

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[10] The exact physiological rôle of the ICSH factor in this relationship is under investigation.

THE RÔLE OF THE CERVICAL SYMPATHETICS IN THE REGULATION OF THYROID AND THYROTROPIC FUNCTION

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THE INTERRELATIONSHIP of the thyroid and the sympathetic nervous system has been subjected to numerous experimental studies, chiefly because of the many sympathicotonic features present in thyrotoxicosis. Nevertheless, the results have been conflicting, as is evident from the reviews of Marine, Reid and Holman, and Means (1, 2, 3).

In the light of our present knowledge, it is clear that an investigation of the interrelationship of thyroid and sympathetic nervous system must consider at least three theoretical possibilities.

a), *The question of a sympathetic secretory innervation of the thyroid.* There is at present no convincing or direct proof of such an innervation. When critically considered, the results of numerous histological studies, especially after unilateral cervical sympathectomy or sympathetic stimulation, indicate that no changes are produced in the thyroid (4, 5, 2).

When cold stimulation is applied in the absence of the hypophysis, the histological picture of the thyroid is abnormal and the body temperature falls (6). The elevation of basal metabolism after cervical sympathetic stimulation reported by Haney (7) could not be confirmed by Friedgood and Bevin (8). The temporary slight decrease in basal metabolism found by Cannon and his co-workers (9) in cats after cervical sympathectomy was observed to be absent in rats after thoracal sympathectomy by Lee and Bacq (10). A sympathetic vasomotor innervation of the thyroid, as suggested by Nonidez's (11) histological studies, has been supported by direct circulatory experiments (13, 14). In contrast to the above concept, the idea of a so-called 'terminal reticulum' ending in relation with the thyroid cells has been advanced by Sunder-Plassmann (15), although its reality has been denied by Nonidez (12).

b), *The question of interrelated thyrotropic and sympathetic influences on the thyroid.* Manley and Marine (16) and Marine and Rosen (17) have demonstrated that transplanted and intact thyroids are similar and react equally to thyrotropic hormone. Aron and Dobrazaniecki (18) claim that after cervical sympathectomy there is no reaction of the thyroid to thyrotropic hormone, while Pieper (19) states that a reaction is present, though smaller than in controls. Krayer's metabolic and histologic studies failed to show any abnormalities (20). The studies of Uhlenhuth et al. (21) in salamanders suggest that adrenalin and pilocarpine sensitize the thyroid and

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organism to thyrotropic hormone. The exact mechanism of this effect is at present unknown.

c), *The question of an influence of the cervical sympathetics on the thyrotropic function of the anterior pituitary.* Possible evidence in favor of such control has been presented in the experiments of Cannon et al. (22) involving phrenic-sympathetic anastomosis.

The experiments reported in the present paper were devised to investigate in rats certain aspects of the questions proposed above. More specifically they include the following problems.

I. The question of the secretory innervation of the thyroid.
   a) The effect of cervical sympathectomy and cold stimulation respectively on the thyroid in the absence of the anterior pituitary.
   b) the direct effect of cervical sympathectomy on the thyroid in the presence of the anterior pituitary.
   c) the effect of cervical sympathectomy on the reaction of the thyroid after thyrotropic stimulation induced by cold.
II. The effect of cervical sympathectomy on the thyrotropic function of the anterior pituitary.

In the various experiments histological examination utilizing the average cell height of the follicular epithelium was used as a quantitative index of thyroid activity. This method permits study of the activity of both thyroid lobes respectively and independently from the reaction of the peripheral organism. Furthermore, it is a sensitive indicator for the thyrotropic hormone.

MATERIAL AND METHODS

One hundred and forty-five male rats were used, varying in weight from 120 to 130 gm. One hundred and three of these were used as experimental animals and 42 as controls.

The experimental procedures included unilateral and bilateral cervical sympathectomies and hypophysectomies in different combinations. Many animals were subjected to cold (5° to 6° C.) as a stimulant of the sympathetic system and of the thyrotropic activity. The exact procedures and numbers of animals used with each experiment are presented under 'Results.'

All operations were performed under ether anesthesia. In cervical sympathectomies the upper ganglion was removed, together with the chain down to the lower ganglion. In some instances removal of the middle ganglion was not accomplished because of operative difficulties. Only those animals showing a marked enophthalmos after sympathectomy were used. Hypophysectomies were performed according to the Smith-Selye technic, and the completeness of the operation was carefully controlled under the binocular dissecting microscope. Whenever 'blank operations' are referred to in the paper, it means that the neck of the rat was opened and the tissue handled without removing or cutting the cervical sympathetics.

After killing by decapitation, the thyroid and adrenal glands were weighed and fixed in formol. 5μ sections through the middle half of the thyroid were stained with haematoxylin and eosin and examined with a uniform technic. This technic involved the measurement of the height of one average-sized cell from each of about 100 successive follicles encountered in a typical cross section of each thyroid gland. The average cell height so obtained was taken to be the Cell Height Index (CHI) of thyroid activity. Other marks of secretory activity, such as secretion vacuoles, the amount and staining of colloid, were utilized as supplementary information. All measurements were taken with a Spencer ocular micrometer.

RESULTS

Hypophysectomy alone and with bilateral sympathectomy was done in order to investigate any possible supporting rôle of the cervical sympathetics on the thyroid after hypophysectomy.

Hypophysectomy with intact thyroid innervation. Twenty-one hypophysectomized rats, as compared with 10 unoperated controls, showed a marked decrease of thyroid activity as early as the fourth to fifth post-operative day, as evidenced by the CHI (see fig. 1, crosses, curve *A*). The CHI continued to fall until the 9th day, after with it remained fairly constant. The weights of the thyroid and adrenals were also decreased.

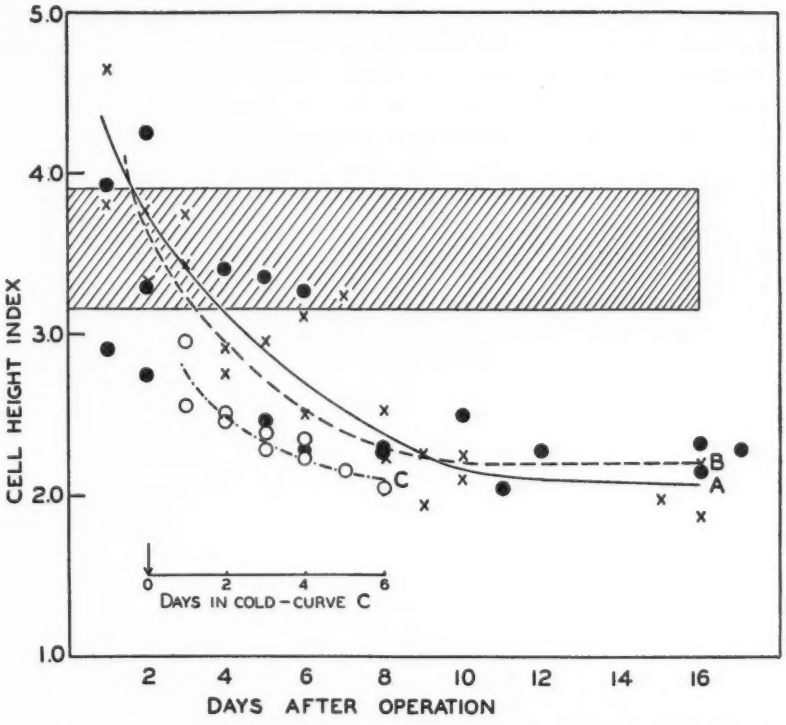

Fig. 1. Effect of hypophysectomy on the CHI. Curve *A*—Effect of hypophysectomy (crosses). Curve *B*—Effect of hypophysectomy and bilateral cervical sympathectomy (black dots). Curve *C*—Effect of hypophysectomy and cold stimulation (circles). Below Curve *C*, the time is indicated during which the animals were exposed to cold. The shaded area represents the CHI variation in unoperated control animals.

Hypophysectomy with bilateral cervical sympathectomy. Experiments on 18 rats showed that the effect on the CHI of these two operations together is approximately the same as that of hypophysectomy alone (see fig. 1, black dots, curve *B*). Although the initial fall in CHI is slightly steeper than in hypophysectomized animals, the final results to not differ significantly.

Hypophysectomy with intact thyroid innervation and cold stimulation. In these experiments an attempt was made to stimulate with cold any possible sympathetic secretory nerves to the thyroid after elimination of anterior pituitary effects.

Although 20 experimental rats were used, the mortality in the cold room was high, and only one lived as long as 6 days. A comparison of the resulting curve (fig. 1, circles, curve C) with the pure hypophysectomy curve shows that cold does not stimulate any thyroid reaction in hypophysectomized animals, even if the sympathetics are intact. The fact that the pure hypophysectomy curve is slightly higher than that of hypophysectomy with cold might be attributed to the warm season in which the latter experiments were

TABLE 1. A. THE EFFECT OF LEFT CERVICAL SYMPATHECTOMY ON THE CHI OF EACH THYROID LOBE

Rat no.	Days after cervical sympathectomy	CHI L	CHI R
173	1	2.34	2.22
174	1	3.30	3.37
176	4	3.05	2.98
175	5	2.56	2.51
177	6	2.67	2.74
179	8	2.80	2.80
180	9	3.02	2.86
181	12	2.60	2.66
183	15	3.14	3.04

B. THE EFFECT OF INCREASED THYROTROPIC ACTIVITY (COLD STIMULATION) ON THE CHI OF EACH THYROID LOBE AFTER LEFT CERVICAL SYMPATHECTOMY AND ISTHMUS SECTION

Rat no.	Treatment	CHI L	CHI R
460	3 days after left cervical sympathectomy with isthmus section, subjected to cold for 7 days	6.81	7.00
461		6.80	6.37
462		5.53	5.77
463		4.59	4.51
464		5.70	5.68
		Ave. 5.88	Ave. 5.86

performed, with a resulting lower initial level of the CHI. The adrenal glands were atrophic and similarly failed to show any cold reaction.

Left cervical sympathectomy with sectioning of thyroid isthmus. These experiments were designed to study any possible local effects of sympathectomy on the ipsilateral thyroid.

In 9 experimental animals the CHI of the left and right thyroid lobes was determined 2 to 15 days after unilateral sympathectomy. The differences between the two lobes (maximally 0.16 μ, average 0.08 μ) are within the limits of experimental error (see table 1, A). The CHI of one lobe had no consistent dominance over that of the other. However, unilateral sympathectomy results in a slight and temporary hypoactivity in both thyroid lobes (fig. 2, curve *B*).

Left cervical sympathectomy and sectioning of thyroid isthmus and cold stimulation. Since the evidence we have obtained indicates that the cold reac-

tion of the thyroid is mediated through the anterior pituitary, these experiments test any possible effects of sympathectomy in interfering with the action of thyrotropic hormone on the thyroid. These experiments also serve as controls for the experiments involving bilateral sympathectomy and cold.

Five rats were placed in the cold room for 7 days, starting on the third post-operative day. The resulting cold reaction of both lobes (see table 1, B) was of the same order as in 5 blank operation controls (CHI—5.86 *vs.* 5.75 in

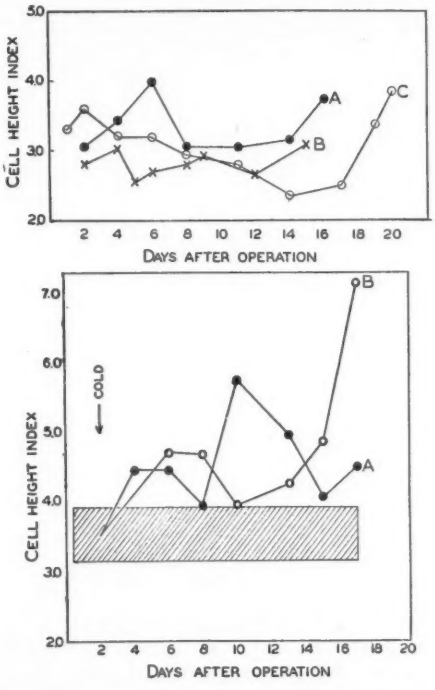

Fig. 2. EFFECT OF SYMPATHECTOMY ON THE CHI. Curve *A*—Effect of blank operation (black dots). Curve *B*—Effect of left cervical sympathectomy (crosses). Curve C—Effect of bilateral cervical sympathectomy (circles). Fig. 3. EFFECT OF BILATERAL CERVICAL SYMPATHECTOMY ON THE COLD REACTION OF THE THYROID. Curve *A*—Blank operation and cold stimulation (black dots). Curve *B*—Bilateral cervical sympathectomy and cold stimulation (circles). The shaded area represents the CHI variation in unoperated control animals.

controls). There was no significant difference in the CHI of the two lobes (see table 1, B), and no consistent dominance of one lobe over the other.

Another series of 5 rats exposed to cold for 3 days, starting on the 6th post-operative day, gave similar results.

These experiments indicate that cold (thyrotropic) stimulation affects equally the intact and sympathectomized lobes of the thyroid.

Bilateral cervical sympathectomy. Twenty experimental rats, as compared with 12 blank operation controls, show that as a result of bilateral sympathectomy the thyroid exhibits a temporary hypoactivity which is

most marked 14 to 16 days after operation. By the 20th post-operative day the CHI is again normal (fig. 2, curve C). Blank operation controls showed a small post-operative increase 4 to 6 days after the operation. The weights of the thyroid and adrenals were not changed.

Bilateral cervical sympathectomy with cold stimulation. These experiments were done to control the results of the foregoing study, and also to show whether bilateral sympathectomy can prevent the characteristic cold reaction of the thyroid mediated through the anterior pituitary.

Fifteen experimental and 15 blank operation controls were kept in a cold room for 2 to 17 days, starting 2 days after the operation. As shown in figure 3, the initial rise and subsequent decline in CHI in experimental and control animals are similar during the first week, after which the two groups react differently. The control group (curve *A*) shows a sharp second rise in CHI after 9 to 14 days in the cold, with a rapid fall following a day or two later. On the other hand, in the experimental animals (curve *B*) the initial rise is followed by a more delayed fall, which in turn changes to a very pronounced increase in CHI occurring on the fifteenth to seventeenth day of cold, and reaching a height far exceeding the maximal CHI of the control group. The fall after the initial rise in the experimental group occurs at the same time, and seems comparable to the similar fall seen in the bilaterally sympathectomized rats which were not stimulated by cold (cf. fig. 2, 3).

DISCUSSION

I. *The question of a sympathetic secretory innervation of the thyroid.* In the foregoing experiments the possibility of a sympathetic secretory innervation of the thyroid has been investigated from several angles with negative results.

Stimulation of the animal by cold does not produce any histological reaction in the thyroid after hypophysectomy, even if the thyroid sympathetic innervation be intact. These experiments agree with Wolff and Greep (6), and serve as additional evidence that the response of the thyroid to cold depends on the thyrotropic function of the hypophysis.

Furthermore, the cervical sympathetic has no histologically detectable supporting rôle on the thyroid in hypophysectomized animals, as is evident from the identity of the CHI curves after hypophysectomy alone, and after hypophysectomy with sympathectomy.

Unilateral cervical sympathectomy with isthmus section failed to produce any difference between the CHI of the two lobes, even after pronounced thyroid stimulation (3 to 6 days' exposure to cold). These studies suggest that sympathectomy does not produce any direct histological thyroid reaction, or any histological changes in the reaction of the thyroid to increased thyrotropic stimulation produced by cold. The evidence derived from these studies, as well as from the earlier experiments cited in the introduction regarding sympathetic secretory innervation of the thryoid, is essentially negative. However, the question is complicated by the known

vasomotor innervation of the thyroid and by the influence of the cervical sympathetic on the anterior pituitary.

II. *Cervical sympathetics and thyrotropic function of the anterior pituitary.* Bilateral sympathectomy is characteristically followed by a delayed mild fall in thyroid function which is succeeded by a compensatory rise. This type of hypoactivity is noticeable in both thyroid lobes even after unilateral sympathectomy, and is more marked after bilateral sympathectomy with cold stimulation. Since unilateral cervical sympathectomy does not produce any more of a reaction in the ipsilateral thyroid lobe than in the contralateral lobe, the characteristic effect of bilateral cervical sympathectomy on the thyroid must, according to our present knowledge, be mediated through the anterior pituitary. Although bilateral sympathectomy temporarily depresses the thyrotropic function of the anterior pituitary and modifies the cold reaction, these transient effects of sympathectomy are subsequently compensated through other pathways. Consequently, the cervical sympathetics appear to be unnecessary for continued histologically normal thyroid function.

The feeble and transient decrease of thyroid function demonstrated histologically after sympathectomy in rats is interesting in view of the slight changes in basal metabolism as reported by Cannon et al. (9) in cats, although the results of Lee and Bacq (10) in rats after thoracal sympathectomy were negative. It should be recalled also that a possible influence of the cervical sympathetics on the thyrotropic function of the anterior pituitary is suggested by the phrenic sympathetic anastomosis experiments of Cannon et al. (22).

The cervical sympathetics seem to bear to the thyrotropic function of the anterior pituitary a relation somewhat similar to that born to the gonadotropic function. Although sympathectomy does not prevent ovulation after mating, fertilization or delivery (9, 23, 24, 25), certain other experiments indicate that it can prevent pseudopregnancy (24) or diminish its frequency (26) after electric or glass rod stimulation of the cervix.

SUMMARY

The anterior pituitary is the principal activator of the thyroid. Hypophysectomy in rats is followed by thyroid atrophy which cannot be compensated by sympathetic stimulation (cold). This indicates that the cold reaction of the thyroid is mediated through the anterior pituitary. Unilateral cervical sympathectomy in the rat neither affects the ipsilateral thyroid lobe nor modifies the histological evidence of thyroid activity after thyrotropic stimulation. The present experiments do no speak for a sympathetic secretory innervation of the thyroid.

Bilateral cervical sympathectomy in rats causes a temporary and mild hypoactivity of the thyroid, apparently mediated through the anterior pituitary. Similarly, sympathectomy modifies the cold reaction temporarily. These transient effects of cervical sympathectomy are subsequently com-

pensated through other pathways. The cervical sympathetics appear to be unnecessary for a continued histologically normal thyroid function.

The author wishes to express his gratitude to Dr. George B. Wislocki for his interest and kindness during the work, and to Dr. H. Stanley Bennett who corrected the English.

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ACTION OF ESTRIN ON THE BLOOD PRESSURE OF THE ANAESTHETIZED CAT

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THERE HAVE BEEN a number of papers in recent years discussing the possible influence of estrin on blood pressure. Daniel, Crainicianu, and Mavromati (1) reported drops in blood pressure as great as 50 mm. of Hg lasting an hour or longer following the intravenous injection of 1 cc. of menformon (Hogival), (highly purified estrogen), in women. They stated that no drops occurred in men or male cats, whereas an immediate hypotension was obtained in female cats. Crainicianu (2) confirmed this work using 100 U.S. (mouse units) of folliculine (Hogival) intravenously in human beings. Melchionna (3) obtained no change in the blood pressure of 9 dogs under nembutal or ether anaesthesia from the intravenous administration of 250 R.U. of crystalline theelol dissolved in 0.25N sodium hydroxide diluted with saline. Ayman (4), in a careful clinical study observed no effect of theelin on the blood pressure of 10 patients even after treatment over a period of months; the largest doses, however, totalled only 300 R.U. by mouth, along with 50 R.U. subcutaneously per day. Other investigators also differ. Mayrhofer (5) and Fellner (6) reporting drops in the blood pressure of hypertensives treated with menformon and Wallis (7) reporting negative results with perlatin. The present work was undertaken in an effort to ascertain the effect of large doses of crystalline estrin on the blood pressure of anaesthetized animals having a suitable basal blood pressure.

METHOD

A total of 17 adult cats was used. Of these, 6 were normal males, 2 were castrated males, 3 were normal females, and 6 were spayed females. The spayed females were used within 8 days of operation to prevent too great atrophy of the uterus. The animals were anaesthetized with dial (Ciba) given either by stomach tube (0.8 cc. per kg. of body weight), or by intraperitoneal injection (0.7 cc. per kg.). They were prepared by denervation of one hind leg by cutting the sciatic, femoral, posterior and lateral femoral cutaneous, and obturator nerves as high up as possible. In several cases one uterine horn was split to expose the endometrium and sutured into the abdominal wall. Continuous blood pressure readings were recorded by means of a carotid cannula connected to a Hurthle manometer. Due to the small exchange of fluid with this method, it was frequently possible to record continuously for 8 hours without a clot. Crystalline estrin in oil[1] was injected intramuscularly into the dener-

[1] We are indebted to Dr. E. A. Sharpe of Parke, Davis & Co. for the generous quantities of both theelin in peanut oil and theelin in aqueous solution which he supplied to us.

vated leg in doses varying from 1000 to 10,000 I.U. Estrin in aqueous solution (200 I.U. per cc.) was injected slowly into the femoral vein in doses of from 800 to 2000 I.U. The animal's temperature was maintained constant throughout the experiment by means of an electric heating pad.

RESULTS

An animal prepared in the above fashion frequently maintained a mean blood pressure of approximately 150 mm. of Hg for over 24 hours.

Estrin in Aqueous Solution

The slow intravenous injection of estrin in aqueous solution at body temperature was followed by a prompt rise of blood pressure which varied (10 to 40 mm. of Hg) in proportion to the quantity injected. This was fol-

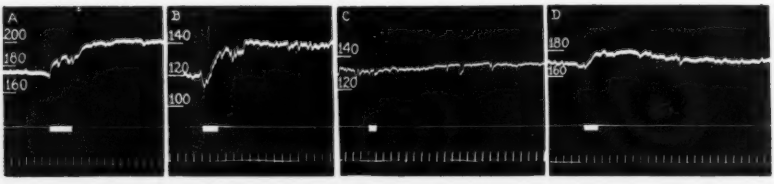

Fig. 1. *A.* Intravenous injection of 1000 I.U. estrin in aqueous solution (5 cc.). Time interval 30 sec. *B.* Intravenous injection of 5 cc. 0.005N sodium hydroxide. *C.* Intravenous injection of 5 cc. 0.005N sodium hydroxide plus 44 mg. crystalline sodium chloride. *D.* Intravenous injection of 1000 I.U. estrin in aqueous solution (5 cc.) plus 44 mg. crystalline sodium chloride.

lowed by a subsequent slow rise in some cases to a total of 50 mm. of Hg above the original pressure. This rise in blood pressure lasted from 30 minutes to more than two hours, gradually returning to the original pressure. No subsequent compensatory fall below the initial pressure was noted (fig. 1-A).

Controls. A control solution of the solvent for aqueous estrin containing 0.5% ethyl alcohol and sufficient sodium hydroxide to make it 0.005N was prepared.[2] An equal volume of this solution warmed to body temperature and similarly injected caused a rise of blood pressure practically identical in magnitude and time course with that from aqueous estrin. A rise in blood pressure could likewise be obtained with 0.005N sodium hydroxide alone (fig. 1-B). When crystalline sodium chloride was added first to the control solution and then to aqueous estrin in quantities sufficient to make them approximately isotonic (i.e., 44 mg. per 5 cc.), the rise in blood pressure vanished or became minimal and transitory (fig. 1-C, 1-D).

Estrin in Oil

Estrin in oil in 1 cc. amounts injected into the thigh muscles of a leg with its nerve supply intact quite regularly but not invariably caused a slow steady drop in blood pressure ranging from 30 to 40 mm. of Hg over a period of one to three or four hours. This returned slowly in a number of cases to

[2] We are indebted to Dr. Oliver Kamm of Parke, Davis & Co. for the formula of the solvent for aqueous theelin.

approximately the original level. The dose of estrin, whether 1000 or 10,000 I.U., did not seem to be significant in either the size or time relationship of this fall. Similar amounts of estrin in oil injected into the denervated leg caused no alteration in the blood pressure. In one animal in which an injection on the denervated side caused a drop in pressure, manipulation of the leg alone also caused a drop, possibly due to an incomplete denervation. Absorption of the estrin was indicated by a change in the color of the exposed uterine endometrium, when available, from a yellowish gray translucence to a definite pink and in some cases to a red. It is interesting that no change in the color of the endometrium was observed after the intravenous injection of estrin in aqueous solution.

Controls. One cc. of peanut oil, the solvent in estrin in oil, injected into a leg with the nerve supply intact caused a drop in blood pressure entirely similar in size and time course to that produced by estrin in oil in an innervated leg.

DISCUSSION

Pompen (8), MacLeod and Reynolds (9), and others have shown that the absorption of estrin from oily solution is quite prompt, occurring certainly within one to two hours following injection. These findings are in accord with observations made on the exposed endometrium in the above experiments. The fact that no change in the blood pressure was noted in from 2 to 5 hours following injection does not preclude the possibility of a late effect of the hormone. Spontaneous variations of blood pressure, though minimal in cats under dial anaesthesia at a constant temperature, place such possible late effects beyond the limits of this experiment. The changes in blood pressure that were noted within the time limits of the experiment were found to be due in each case to the solvent for the hormone. The mechanism by which these changes were produced is not entirely clear. The rise in pressure following aqueous estrin may have been due to hemolysis since it was also observed after injections of distilled water alone and was absent after the solution was made approximately isotonic with sodium chloride. The drops following injections of estrin in oil into an innervated leg may have been due to irritation of sensory nerves by the presence of the oil. Weak afferent stimuli are known to cause a drop in blood pressure even in completely sympathectomized animals (10). Drops in blood pressure entirely similar to those produced by estrin in oil were obtained with the same oil without the hormone. It was concluded, therefore, that estrin by itself had no effect on the blood pressure of anaesthetized cats within the time limits of the experiment. The sex of the animal used was likewise found to have no significance, contrary to the results of Daniel, Crainicianu, and Mavromati (1). It must be remembered that the cats used in these experiments were normal animals. It is possible that estrin might have an entirely different effect in the presence of a pre-existing hypertension.

SUMMARY

Estrin in aqueous solution injected intravenously into cats under dial

(Ciba) anaesthesia gives an immediate prolonged rise in blood pressure of 10 to 50 mm. of Hg, which is absent when the solution has been made approximately isotonic with crystalline sodium chloride prior to injection.

Estrin in oil solution injected intramuscularly into the same animal produces a slow drop in blood pressure of 30 to 40 mm. of Hg over several hours which does not occur when the injection site has been previously denervated.

Both effects on blood pressure are obtained equally well when the solvents without the estrin are used. Estrin by itself has no effect on the blood pressure of anaesthetized cats. The sex and presence or absence of gonads in the experimental animal are not significant for these findings.

We are indebted to Dr. Joseph C. Aub for many valuable suggestions in the preparation of this paper.

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THE CORTICAL ALPHA RHYTHM IN THYROID DISORDERS[1]

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THE REGULARITY and constancy of the 10-cycle 'alpha rhythm' obtained from the posterior part of the human skull, first described by Berger in 1929 (1), is one of the most striking phenomena brought out by electroencephalography. In an effort to understand what factors determine this rate, Rubin, Cohen, and Hoagland in 1937 (2) found that the hyperthermia, used in the treatment of general paresis, increased the number of alpha waves per second. They found thyroxin did the same thing. Jasper and Andrews in 1938 (3) experimentally obtained identical results. Lindsley and Rubenstein in 1937 (4) found a remarkably high coefficient of correlation between alpha rate and a total caloric output per hour, although that between alpha rate and basal metabolic rate proper was rather low. It would seem apparent from the work of the authors cited, however, that the mean alpha rate is connected in some way with the rate of oxygen consumption in the body as a whole, and it accordingly seemed worth while to extend the observations into both higher and lower ranges of metabolic rate, using for the purpose the material made available by the Thyroid Clinic at the Massachusetts General Hospital.[2] It has in this way been possible to follow the alpha rates in cases of hyperthyroidism before and after iodinization and thyroidectomy, and also in hypothyroid patients during the course of substitution therapy.

The procedure was simply to determine the alpha rate in a patient with thyroid disease who had recently had a B.M.R. determination. A 2-channel ink-writer oscillograph with push-pull amplifiers was used for the purpose of recording the electroencephalogram, one channel being provided with a band-pass filter, adjustable for various frequencies, so that if the alpha rhythm were not prominent in the unfiltered record the masking higher and lower frequencies might be suitably attenuated. The head leads most commonly used were a right occipito-parietal lead with a ground connection to the lobe of the right ear. A number of trains of alpha waves were selected from the record and the rate for each train was estimated by dividing the number of waves by the duration in seconds. Long trains were, of course, preferred for this purpose, whenever they were available. The unfiltered

[1] Part of this work was aided by a Grant from the Milton Fund of Harvard University.

[2] In this connection the authors wish to express their gratitude to Drs. J. H. Means and Jacob Lerman for their interest and suggestions, and to the staff of the Metabolism Laboratory for their cooperation throughout this series of experiments, and to Dr. Stanley Cobb and Dr. James B. Ayer for their helpful suggestions.

record was also given preference, but the filtered one was used whenever a heavily masked alpha rhythm in the normal record made wave-counting difficult. The average for the whole run was calculated when, or if, an adequate number of short-period rates had been obtained.

When the work first started the patients who had had B.M.R. determinations that day were brought down from the ward in a wheel chair, usually around mid-forenoon or mid-afternoon, and the electroencephalograms were obtained with no preliminary periods of preparation or rest. A little later on, however, it occurred to us that as long as two rates were to be compared—one an alpha rate and the other a specially determined rate of oxygen consumption—one might reasonably expect the correlation, if any existed, to be more readily demonstrable if both rates were measured under the same standardized conditions. The routine was accordingly modified so that this could be done. The patient was sent down to the metabolism laboratory (or came in as an out patient) early in the morning in the fasting condition, and after the usual half-hour period of rest the two 6-minute spirometer tests were made. When a satisfactory spirogram slope had been obtained the patient was lifted, still supine, onto a litter and wheeled to the electroencephalographic laboratory, where the alpha rate was determined with the patient still lying on the litter. After the electroencephalographic run the patient was returned to the metabolism laboratory for weighing and computation of the B.M.R. In most of the out patient cases only one determination was possible in each patient, but in many of the ward patients several runs were obtained, including the periods before and after thyroidectomy or thyroid administration.

According to the circumstances of the individual test, a patient's average alpha rate was placed in one of two categories, called 'reliable' and 'questionable' respectively. It might happen that a patient would, through a slip-up in instructions, get up out of bed before having the electroencephalogram made; or perhaps he would ill-advisedly eat breakfast before coming in for his out patient test; or again, he might produce an electroencephalogram with so few trains of alpha waves that the final average would be looked upon with suspicion. In such cases the alpha rates were classed as 'questionable.' All nonfasting rates were considered 'questionable,' quite apart from the character of the electroencephalogram or the success realized in adhering to the prescribed routine. The 'reliable' group included only those who had had a normal fasting B.M.R. determination, who had been brought to the electroencephalographic laboratory without being allowed to get out of bed, and who finally produced a brain-wave record from which the alpha rate could be easily measured.

RESULTS

In view of these rather laborious precautions the results are not entirely without humor as well as interest. It appears that the more careful one is in having the patient at basal conditions for the alpha-rate determination, the more remote are one's chances of obtaining a good, or even only fairly good, coefficient of correlation. The results are shown in tabular form in table 1.

TABLE 1. COEFFICIENTS OF CORRELATION BETWEEN ALPHA RATE AND OXYGEN CONSUMPTION

Rates being compared	*'Reliable'* (basal with strong alpha)	*'Questionable'* (either not basal or with weak alpha)	*'Combined'*
Alpha rate and B.M.R.	0.392	0.781	0.668
Alpha rate and total colories per hour	0.127	0.737	0.594
Alpha rate and cal. per hour per sq. meter	0.136	0.723	0.601

The coefficient of correlation between the alpha rate and B.M.R. is large enough in relation to the probable error to be highly significant.

TABLE 2.

	Basal (17)	Not Basal (26)	Combined (43)
'Questionable' alpha rate and B.M.R.	0.421	0.883	0.781

It will be noted that in each case the 'questionable' group shows a much better coefficient than the 'reliable' one. The best correlation in the series is that between alpha rate and B.M.R., although those between it and total calories per hour and total calories per square meter per hour are nearly as high. In this respect our results differ considerably from those of Lindsley

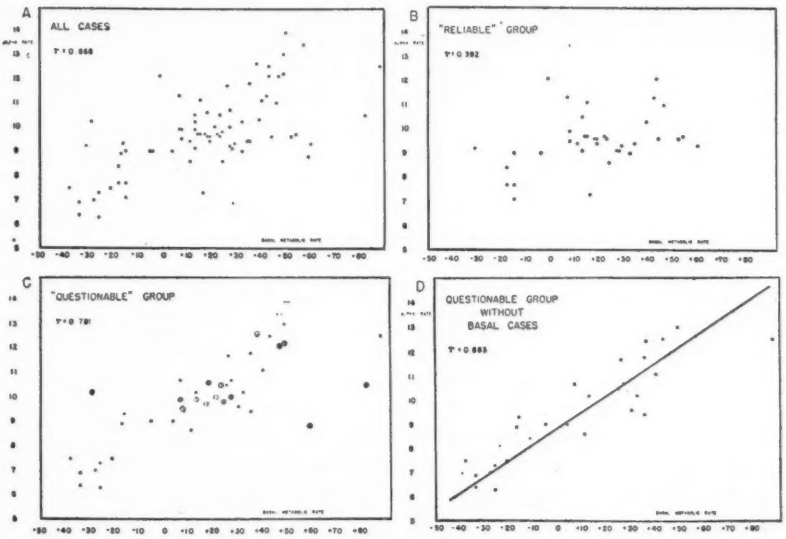

FIG. 1. BASAL METABOLIC RATES, AS ABSCISSAE, PLOTTED AGAINST ALPHA RATES, BASAL AND OTHERWISE, AS ORDINATES. *A*, The complete mixed group of 80 determinations. $r = 0.668$ *B*, The 'reliable' group (see text) of 37 determinations. All alpha rates taken under basal conditions. $r = 0.392$. *C*, The questionable' group (43 points), with basal cases shown as dots-and-circles and the others as solid dots. $r = 0.781$. *D*, The 26 determinations of the 'questionable' group which remain after the 17 basal ones have been removed. The line of regression, as determined by the method of least squares, has been drawn in. $m = 14.89$; $r = 0.883$.

and Rubenstein (4), for although their coefficient (0.396) for basal alpha rate and B.M.R. is practically the same as ours, they obtained a much higher coefficient (0.903) when they compared basal alpha rate and total calories per hour. The reason for this tremendous discrepancy is not yet clear.

The most accurate relationship occurred in the myxedema group; cor-

relation coefficient 0.9, and least accurate relationship in the hyperthyroids 0.51.

However that may be, it seems evident that if our data are significant there must be some part of the procedure of running an electroencephalogram under basal conditions that has a strong tendency to upset an otherwise fairly consistent alpha rate. In this connection it was recalled that many of the runs in the 'questionable' group were also made under basal conditions, but were placed in this class because of supposed unreliability of the alpha determination, or for some other reason. Coefficients of correlation were therefore calculated for the 'questionable' class after dividing it into two sub-groups, in one of which the conditions were basal and in the other not. These coefficients, as shown in table 2, demonstrate that the r for the 'questionable' group is still further increased by removing the basal cases from it, whereas the coefficient for the removed basal cases is about the same as that for the 'reliable' basal group.

A few incidental observations are perhaps worth recording. One is that in any given patient whose B.M.R. is being therapeutically altered, the alpha rate does not necessarily change in the same direction as the B.M.R. It is difficult to obtain accurate data on this point, for the number of determinations on any one patient was never very large—the highest being 9, which was exceptional. Moreover, the patients in whom repeated tests were possible were nearly all ward cases, and it was with these that most care was taken; consequently most of them fall into the 'reliable' group and scattering of the points is prominent. Nevertheless, one can get a rough idea as to the general direction of a straight line drawn so as to pass through or near a majority of the points in any one case, and if this is done in the 15 patients in whom repeated study was possible it is found that the slope is positive in 6 and negative in one, while in the other 8 cases there was not enough variation in the alpha rate to give the line any significant slope one way or the other, although the B.M.R. might vary considerably.

Another finding is that a dominant-alpha patient may change to a mixed or rare one, or vice versa, without any obvious reason for the change; such as, for example, the degree of 'nervous tension' in the patient at the time of the test. There were only 3 cases in which this variability of alpha type was noticed.

DISCUSSION

In a series of patients with thyroid dysfunction (thus providing a rather wide range of basal metabolic rates) the B.M.R. is compared with the corresponding frequency of the cortical alpha rhythm. When all the 80 determinations are considered together, a significant coefficient of correlation (0.688) is found. When the series is divided into two groups, in one of which the alpha rate was determined under basal conditions and in the other not, the coefficient of correlation drops to around 0.4 in the basal group and rises to 0.883 in the non-basal. The explanation for this peculiar behavior is not yet clear, but good alpha rhythm may depend on a relaxed well-fed, satisfied state (5).

It is clear from this study that the electroencephalogram does show some relation to the metabolic state, but since the relationship is not accurately linear, the substitution of the former for the latter is not likely.

It would be reasonable to find increased cortical rhythm with increased metabolic activity. A newer procedure usually is regarded as less reliable than a long tried one. For this reason, we may feel that the lack of perfect correlation lies in the fault of the electroencephalogram, but we must not lose sight of the fact that the B.M.R. is not without its errors, and that abnormal values may be found—especially in the elevated group in frightened or psychoneurotic people. Some of the lack of correlation may perhaps then be blamed on the B.M.R.

The fact that the best alpha values are obtained in patients fed, relaxed, and satisfied, is in keeping with the conception of the nature of the 10-cycle waves.

The data in this paper may lead others, working with the electroencephalogram, to make B.M.R. determinations if the alpha rhythms are far off the 10-cycle found (below 8 or above 11).

SUMMARY

Eighty determinations of the alpha rate of the cortical electroencephalograms in a group of patients with thyroid discorders were compared with the basal metabolic rates (12 myxedema and 22 hyperthyroids).

A good correlation exists in the whole group (0.668), suggesting a correlation of alpha rate with metabolic state of the individual. When the alpha rhythm is taken during a 'basal state' (patient fasting and resting one-half hour quietly), the correlation is not as good as when the patient is well fed, satisfied, and relaxed (0.39 compared to 0.883). It is suggested that when low or high alpha rhythms are encountered, a B.M.R. determination be made.

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ENDOCRINE CONTROL OF THE MOTILITY OF THE MALE ACCESSORY GENITAL ORGANS

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THE MOTILITY of the masculine genital organs and their pharmacology in vitro have been the subject of very few papers. Waddell (1) studied the longitudinal contractions of the vasa deferentia of sheep, dogs, rabbits, rats, and guinea pigs, excised and immersed in an oxygenated bath of Tyrode's or Ringer's solution at 37° to 38°. He also examined the seminal vesicles of rats and guinea pigs (2), the uterus masculinus of rabbits (3), and the prostate of rats, guinea pigs, hogs, cats, and rabbits (4). Spontaneous contractions were found in the deferentia of rats and rabbits, in the seminal vesicles of rats and guinea pigs, and in the uterus masculinus of rabbits, but they were not found in the prostate of rats, guinea pigs, cats, hogs, and rabbits nor in the vasa deferentia of dogs, guinea pigs, and sheep. The deferentia and seminal vesicles of the species studied by Waddell exhibited increase in tonus and rhythmical contractions after the application of epinephrine, ergot, hydrastinine, pilocarpine, strychnine, nicotine, and barium chloride. Pituitary extract (posterior lobe) had no effect in this regard, but was active on the uterus masculinus of the rabbit. The pharmacological reactions of the prostate are similar to those shown by the other two organs, but more tonic in character.

Macht (5) confirmed Waddell's observations on preparations of the vasa deferentia and seminal vesicles of the rabbit and occasionally on the guinea pig. On the other hand, neither longitudinal strips nor rings of the vasa deferentia of dogs, cats, sheep, or bull ever exhibited normal rhythmic movements, but only reacted to drugs by simple contractions, thus confirming the views of Loeb and Nagel. Morphine and the phenanthrene group of opium alkaloids increased the tonus and contractions of the vasa deferentia, seminal vesicles, and ejaculatory ducts; papaverine and the isoquinolin group of opium alkaloids decreased their tonus and inhibited their contraction. With the total opium alkaloids the narcotine and papaverine effects on smooth muscle predominated.

Perutz and Merdler (6–9) and Kofler and Perutz (10) described results, similar to those of Waddell. In vivo the vasa deferentia of dogs, rats, and guinea pigs reacted to injected drugs, and contracted during asphyxia or after excitations of the vagus, nasal mucosa, peritoneum, and small intestine. On the other hand, Perutz and Taigner (11) stated that the vasa deferentia of the rat, in vitro, exhibited spontaneous contractions.

From these reports, it is evident that the deferentia and seminal vesicles are excitable by pharmacological drugs and that they are supplied by motor fibers, both sympathetic and parasympathetic. In the course of a study of

the conditions of migration of the testis, we made a systematic research regarding the contractility of the accessory genital organs and its hormonal control. This study became much more necessary as there were no data in the literature relating to the influence of the sex hormones on the motility of the smooth muscles of the masculine genitalia, whereas there were numerous papers on the contractility of the uterus. We herewith present a more detailed description of the methods and results of our researches on which we have already published some preliminary notes (12–15).

MATERIAL AND METHODS

The work was planned to compare the behavior of the accessory genital organs: the vasa deferentia, seminal vesicles, and prostates, excised from normal, castrated, and sex-hormone injected rats.

Castration and extirpation. Castration was made through a median suprapubic incision, leaving intact the tail of the epididymis and the vasa deferentia. To check the rôle of the surgical trauma, some control rats were unilaterally castrated. Within 2 to 139 days after the operation, the organs were removed from the animal under slight ether narcosis, and stripped of all surrounding tissue. In the case of the vasa deferentia, the blood vessels were almost always detached, thus taking away part of the adventitia. The seminal vesicles were separated from the prostate and sectioned at the base. For the prostate, we chose the cranial lobe (terminology in Parkes, Callow, and Deanesly, 17), also called the coagulating gland, which was carefully separated from its connections with the seminal vesicle. As a rule, the accessory genital organs of one side were removed and the animals kept alive for further study of the organs on the other side after some days. In some cases, the same animal first functioned as a normal and later as a castrate. In 63 experiments, comparative studies were made on organs after storage in Locke's solution at refrigerator temperature for varying lengths of time.

Saline bath and myography. The organs, immediately after removal, were transferred to a beaker, which contained Locke's, or at times Tyrode's solution kept at a constant temperature, 37° to 39° C. A stream of oxygen was constantly maintained; the pH of the solution, according to the case, varied from 7.2 to 8.0. Only the longitudinal contractions were registered due to the small size of the organs; one end of the organ was fixed at the bottom of the beaker and the other end attached by means of a thread to the lever of the myograph, which had a load of 0.5 gm. and an amplification index of 6.

In the first experiments only one organ was used, but in most cases we mounted, at the same time and in the same beaker, 2 to 3 organs, belonging to animals under different experimental conditions. Since at the moment of checking the record, the surrounding conditions, temperature, drug concentration, myographic load, and amplification, etc., were strictly identical, the differences in behavior could be observed most critically.

Drugs. The drugs, diluted in 1 to 2 cc. of the saline solution, were injected by means of a lateral tube in the beaker; by pumping the liquid, the solution was almost instantaneously homogenized. The capacity of the beaker varied from 35 to 75 cc., the liquid being frequently changed. After the appli-

cation of a drug, the bath was changed more than once, except in special cases, when there was an interest in the association of various drugs. The quantities to which we refer in this paper represent the total concentration of the drugs in the saline bath.

Treatment with hormones. Groups of rats were treated with estradiol benzoate, progesterone, androsterone, testosterone and its propionate. With the exception of estradiol, all others are synthetic products.[1] The substances, dissolved in 0.5 or 1.0 cc. of sesame oil, were injected subcutaneously every 3 or 5 days, the treatment beginning, as a rule, immediately after castration, The implantation of small tablets of testosterone under the skin as described by Deanesly and Parkes (18) was also used.

RESULTS

The behavior of the vasa deferentia, seminal vesicles, and prostates were very uniform; the modifications detected paralleled the experimental intervention in the donor animals, and followed a general type. The experiments were made immediately after the removal of the organs from the animal.

Behavior of Vasa Deferentia, Seminal Vesicles, and Prostates of Normal and Castrated Rats

An inspection of table 1 shows that spontaneous contraction of these organs after immersion in Locke's solution was very rare in organs from normal and hemicastrated rats. In fact in only one of 31 normal animals and in one of 11 hemicastrates, were spontaneous contractions observed. In both of these cases the indications are that male hormone secretion was subnormal because the seminal vesicles were below normal in size.

In complete castrates, spontaneous contractions of vasa deferentia were observed in over 80% of the 52 animals. Similar reversals were observed in the spontaneous contraction of the seminal vesicles and prostates.

The results described below on the action of drugs are based on the study of 31 vasa deferentia of normal rats, 11 of hemicastrates, 2 of vasoligated, and 52 of complete castrates. The vasoligated and the hemicastrates behaved like normals. The general effects are also given in table 1.

Pilocarpine hydrochloride (1:30,000 *to* 1:60,000). Doses, which would be sufficient to stimulate the vasa deferentia of castrated rats were, usually, inactive to the normal rats. There was also a very conspicuous qualitative difference. In the vasa deferentia of castrated rats there were rhythmic contractions without the tonic type, but in the organs of normal rats, the response was a tonic elevation. The effect on castrated rats lasted longer and often persisted after the change of bath. In the organs with spontaneous contractions, the frequency may also increase.

Acetylcholine hydrochloride (1:30,000 *to* 1:400,000) Acetylcholine was a better stimulant than pilocarpine for the normal rats, but the effect on castrated rats was also stronger and more constant, and had a rhythmic char-

[1] We are grateful to the following firms: Schering-Kahlbaum (Berlin), for the donation of estradiol benzoate (progynon); progesterone (proluton); and the androgenic substances androsterone, testosterone, and testosterone propionate and to Ciba (Basel) for androsterone, testosterone, and testosterone propionate (perandren).

acter; the rhythmic movements lasted much longer and persisted even after the change of the bath.

Physostigmine sulphate (1:400,000 *to* 1:2,000,000). This substance also

TABLE 1. SUMMARY OF THE RESULTS OBTAINED BY THE STUDY IN VITRO OF VASA DEFERENTIA, SEMINAL VESICLES AND PROSTATES OF RATS

Donor	Treatment	Total of organs studied	Total of cases showing automatism	Reactivity to drugs			Type of Contraction		
				Weak	Med.	Strong	Tonic	Mixed	Rhythmic
			Vasa Deferentia						
Normal	None	31	1[1]	18	9	4	20	10	1[1]
Hemicastrate	None	11	1[1]	5	4	2	7	4	0
Vasoligated	None	2	0	2	0	0	2	0	0
Castrate	None	52	42	13	5	34	3	11	38
Castrate	Sesame oil	3	2			3			3
Castrate	Estradiol benzoate	18	11	2	1	15			18
Castrate	Progesterone	14	10		4	10		3	11
Castrate	Androsterone[2]	2	2		1	1		1	1
Castrate	Testosterone[2]	6	2	1		5		3	3
Castrate	Estradiol b.+Progesterone	8	5		2	6		1	7
Normal	Estradiol benzoate	2	1	1		1	1	1	
Castrate	Testosterone or its propionate[3]	18	0	10	6	2	14	2	2
Castrate	Estradiol b.+Testosterone pr.[3]	5	0	5			5		
		172							
			Seminal Vesicles						
Normal	None	12	0	6	3	3	8	4	
Hemicastrate	None	7	0	4	1	2	4	2	1
Castrate	None	19	14		3	16		6	13
Castrate	Estradiol benzoate	9	4		2	7		3	6
Castrate	Progesterone	5	2	1	1	3		1	4
Castrate	Estradiol b.+Progersterone	8	2	4	1	3		1	7
Normal	Estradiol benzoate	1	0			1		1	
Castrate	Testosterone or its propionate	12	0	10	2		10	2	
Castrate	Testosterone pr.+estradiol b.	5	0	4	1		4	1	
		78							
			Prostates						
Normal	None	14	0	10	3	1	10	3	1
Hemicastrate	None	2	0	1	1		1	1	
Castrate	None	17	15	3	2	12	3	4	10
Castrate	Estradiol benzoate	3	3			3			3
Castrate	Progesterone	6	2	1	1	4		2	4
Castrate	Estradiol b.+progesterone	6	2	1	1	4		3	3
Castrate	Testosterone or its propionate	6	0	6			6		
Castrate	Estradiol b.+Testosterone pr.	4	0	3	1		3	1	
		58							

[1] Animals with seminal vesicles of subnormal size.
[2] Treatment insufficient to complete correction of the castration atrophy.
[3] Treatment sufficient to complete morphological normalization of the accessory genital organs.

caused mainly tonic contractions in the normal rats and the rhythmic type in the castrated animals. This drug sensitized the organs for the subsequent action of acetylcholine.

Epinephrine hydrochloride (1:150,000 *to* 1:1,000,000). This caused tonic contractions of the normal vasa deferentia and the mixed or rhythmic type in those of the castrated rat.

Nicotine (1:40,000 *to* 1:80,000). This provoked large ample contractions in the normal organs, followed by a slow relaxation, although faster than after epinephrine. It was also a good stimulant of the castrate organs, producing reactions of the rhythmic, mixed and tonic types. After the application of nicotine, the organs usually were less reactive to other excitants, a phenomenon which would indicate the presence of ganglionic cells. In fact,

ganglionic masses were found in the adventitia of some organs submitted to histological study.

Ergotamine tartrate (*1:80,000 to 1:160,000*) acted like epinephrine, but with a longer latent period and the results were less constant, many organs not reacting at all. After a contact of 8 to 12 minutes, the addition of epinephrine to the bath had no stimulating effect and may have had an inhibiting action on the contracting organs.

Yohimbine hydrochloride (*1:20,000 to 1:40,000*) had a stimulating action. After a contact of 5 to 10 minutes, the action of adrenalin can be reversed, as after ergotamine: absence of excitation or inhibition of the contractions already present.

Barium chloride (*1:5000 to 1:80,000*) produced an elevation of the tonus and very striking rhythmic oscillations in normal organs. In the castrate, the contractions were predominantly rhythmic and it was possible to maintain them for more than one hour.

Pituitrin (0.25 to 3.0 I.U. in 40 cc., Parke, Davis & Co). In almost all cases no effect was produced, either on normal or castrates. In a few cases there were doubtful positive reactions, but only in the castrate. An acetone-powder extract of the bovine posterior hypophysis gave better results, but, in view of the dubious purity of the extract, no conclusion can be drawn.

Atropine sulphate (*1:40,000 to 1:200,000*) inhibited the contractions produced by acetylcholine, pilocarpine, and barium chloride, and at times the spontaneous contractions.

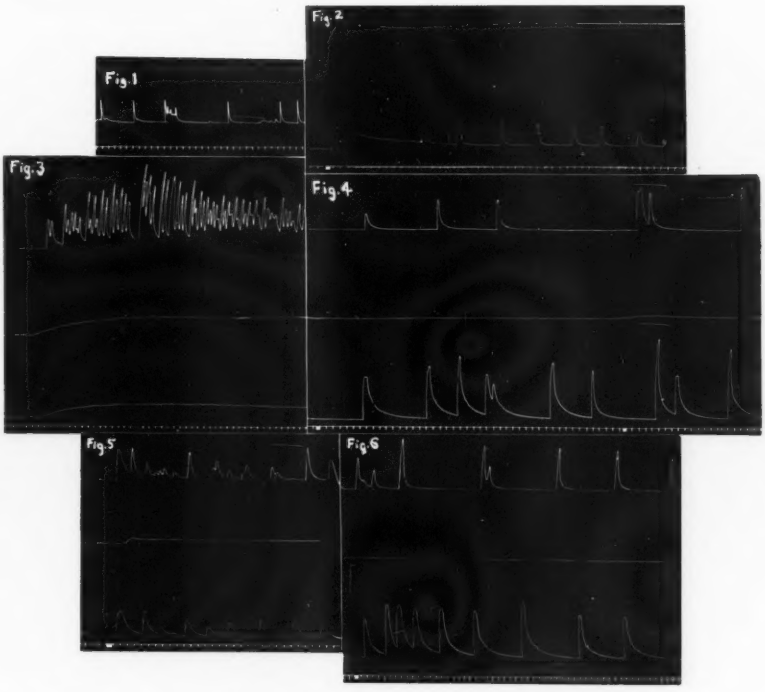
Spermine hydrochloride (*Synthetic, Hoffman-LaRoche—1:10,000 to 1:50,000*), caused inhibition or depression of the movements, both tonic and rhythmic, whether spontaneous or produced by the action of other drugs. The intensity and duration of this effect depended on the respective concentration of the drugs. In most cases, the inhibiting action was not persistent, the movements returned to the lower level after an interval of 1 to 5 minutes. After a previous contact of the organs during 4 to 10 minutes with spermine, the application of stimulating drugs, for instance, epinephrine, acetylcholine, or nicotine, may be completely inhibited.

Morphine hydrochloride (*1:8000 to 1:40,000*) produced variable results, mostly negative.

Papaverine hydrochloride (*1:20,000 to 1:40,000*) had an inhibiting effect on the contractions.

The effects of the drugs and the difference in the behavior of the vesicles and prostates of normal and castrated rats were generally of the same type as those on the vasa deferentia. They were predominantly tonic effects on the normal and rhythmic or mixed on the castrate. However, the reactions were not always as clear cut as with the deferentia. Even in the castrate, it was possible to produce tonic effects but they were less intense than in the normal and were followed by rhythmic oscillations. Occasionally, certain normals reacted in this way, but the predominant type was tonic.

The difference in the records of seminal vesicles and prostates lies, as already mentioned, only in the amplitude; the latter may be considered as miniatures of the graphs of the former.



SIMULTANEOUS RECORDS OF ORGANS SUSPENDED IN LOCKE'S SOLUTION, 37° TO 38° C. TIME MARKS, IN EVERY CASE, 5 SECONDS.

Fig. 1. *Upper.* Right vas deferens of rat 31, 5 days after extirpation of the right testis. *Lower.* Right vas deferens of rat 35, 5 days after bilateral castration. Spontaneous contractions are recorded.

Fig. 2. Effect of epinephrine hydrochloride (1:600,000). Seminal vesicle of rats, 19 days after castration. *Upper.* Rat 82, treated with testosterone propionate (20 mg.). *Lower.* Rat 76, untreated.

Fig. 3. Effect of barium chloride (1:5000). Vasa deferentia of rats, 27 days after castration. *Upper.* Rat 88, uninjected. Weight of organ, 37 mg. *Center.* Rat 86, treated with testosterone propionate (total 17 mg.). Weight of organ, 97 mg. *Lower.* Rat 87, treated with testosterone propionate (17.5 mg.)+ estradiol benzoate (14,500 I.U.). Weight of organ, 86 mg.

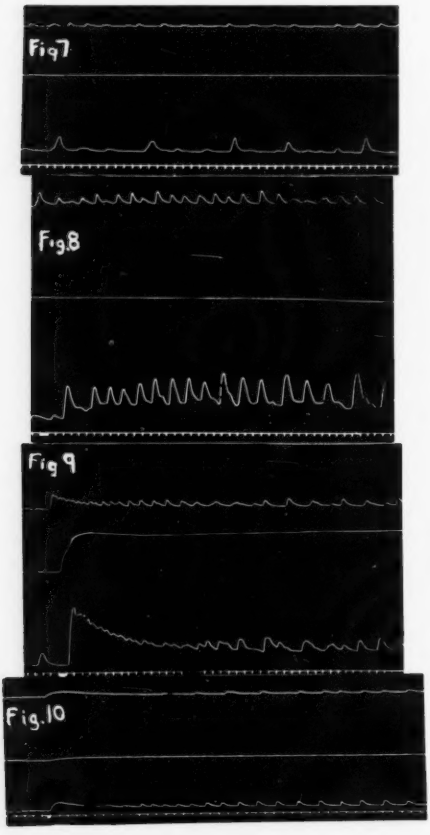
Fig. 4. Effect of pilocarpine hydrochloride (1:120,000), followed by acetylcholine hydrochloride (1:120,000). Vasa deferentia of rats, 15 days after castration. *Upper.* Rat 116, treated with 3000 I.U. estradiol benzoate, followed by progesterone (14.5 mg.). *Center.* Rat 115, implanted with testosterone propionate (3 mg.). *Lower.* Rat 118, untreated.

Fig. 5. Effect of acetylcholine hydrochloride, 1:400,000. Seminal vesicles of rats, 11 days after castration. *Upper.* Rat 107, treated with progesterone (20 mg.). *Center.* Rat 106, treated with testosterone propionate (14 mg.). *Lower.* Rat 105, treated with estradiol benzoate (10 mg.).

Fig. 6. Vasa deferentia 11 days after castration, stored at 4° to 8° C. during 16 hours. Records taken at 37° to 38° C. Effect of acetylcholine hydrochloride (1:35,000). *Upper.* Rat 99, untreated. *Center.* Rat 102, treated with testosterone propionate (22 mg.). *Lower.* Rat 101, treated with estradiol benzoate (9500 I.U.).

The Motility of Vasa Deferentia, Seminal Vesicles and Prostates of Castrated Rats Treated with Sex Hormones

Synthetic androgenic substances. As a test for the activity of the doses used we took the correction of the castration atrophy of the seminal vesicles. If the treatment was sufficient to produce accessories of normal morphology, the contractility of the deferentia, seminal vesicles, and prostates was absolutely normal; that is, absence of automatism, lessened reactivity to the drugs, and especially to those belonging to the parasympathicomimetic group.



RECORDS OF ORGANS SUSPENDED IN LOCKE'S SOLUTION, 37° TO 38°C. TIME MARKS, IN EVERY CASE, 5 SECONDS.

Fig. 7. *Upper*. Cranial lobe of the prostate (coagulating gland) of rat *21*, 119 days after castration. Weight of the organ 18 mg. Spontaneous contractions. *Center*. Seminal vesicle of rat *121*, normal. Weight of the organ 115 mg. No contractions. *Lower*. Seminal vesicle of rat *21*, 119 days after castration. Weight of the organ 40 mg. Spontaneous contractions.

Fig. 8. Effect of pilocarpine hydrochloride (1: 200,000). Same organs of fig. 7.

Fig. 9. Effect of epinephrine hydrochloride (1:2,000,000). Same organs of fig. 7, 8.

Fig. 10. Effect of epinephrine hydrochloride. (1:300,000). Cranial lobe of prostate of rats, 15 days after castration. *Upper*. Rat *116*, treated with estradiol benzoate (2,000 I.U.) and progesterone (15 mg.). *Center*. Rat *115*, implanted with testosterone (2 mg.). *Lower*. Rat *118*, untreated.

Androsterone benzoate in 2.5 mg. total dose divided between 3 injections and distributed over 10 days or 17 mg. in 4 injections spread over 15 days did not alter the castration type of contractility of the vasa deferentia. Testosterone in 20 mg. total dose spread over 9 days, or 30 mg. spread over 13 days, or 3 or 4 mg. of the crystalline material implanted for 10 to 15 days changed the castrate type of accessories to normal as based on the nature of the contractions. The same results were obtained with the injection of a total of 15 to 17 mg. testosterone propionate in 10 to 20 days or by the implantation of 4 mg. of the solid propionate after 11 days. The total number of animals treated is indicated in table 1.

Estrogenic substances with and without progesterone and testosterone respectively. Estradiol benzoate. None of the organs of castrated animals had normal behavior after the injection of 6000 to 100,000 I.U.; on the contrary the characteristics of castration have been exaggerated, the reactions being more typical than those of the uninjected castrate. Of 2 normal animals injected, (3 × 10,000 U in 13 days), the organs of one of them behaved like those of a castrate.

Synthetic progesterone. No effect on the contractile behavior of the accessories of the castrated rat was produced. A previous sensitization with estradiol benzoate did not change the results.

Estradiol benzoate plus testosterone. Three injections of 5000 U of estradiol benzoate with 4 injections of 4 mg. of testosterone benzoate in 18 days or 2 injections of 50,000 estrogenic I.U. plus one injection of 25,000 estrogenic I.U. with the implantation of 2.5 mg. crystalline testosterone in 10 days did not antagonize the effects of the androgen on the contractile behavior of the vasa deferentia, seminal vesicles, and prostate.

Vasa Deferentia and Seminal Vesicles after Storage at Low Temperatures

The vasa deferentia and seminal vesicles maintained, during several days, the contractile reactivity to drugs as measured at body temperature. Positive results have been obtained with deferentia up to the 13th day after removal from the body. No positive reaction was observed with seminal vesicles 6 days after removal from the body.

During the first days, the differences of behavior between normal and castrate may continue unchanged. The deferentia and seminal vesicles of castrates sometimes showed spontaneous contractions when the Locke's solution was warmed at 37°. They reacted well to the drugs and the contractions had a prevailing rhythm of mixed character; they were more sensible to the actions of pilocarpine than the normal or the castrate treated with testosterone. In time, the excitability in all groups decreased, the contractions became slower and the reactivity to the parasympathometic drugs disappeared in the castrate as well as in the normal. However, the reactivity to nicotine, barium, and adrenalin persists longer, the latter was almost always the last to disappear. When the organs did not react to drugs of any sort, they still persistently shortened after addition of some drops of diluted acetic or hydrochloric acids to the medium.

DISCUSSION

In confirmation of earlier authors we found that adrenergic as well as cholinergic drugs had motor effects on the vasa deferentia, seminal vesicles, and prostates of rats. The inhibitory action of spermine is of particular interest because this substance is a normal component of the genital organs and their secretions.

Comparison of the behavior of the organs according to the state of the donor reveals greater excitability of the deferentia, seminal vesicles, and prostates of the castrated animals. It is known that the testicular hormones are absolutely necessary for the normal development of those organs which undergo an extreme atrophy after castration; contrasting with this fact, we

proved that instead of smaller contractility and excitability, the organs of the castrate are more excitable. Those differences may persist even 139 days after castration. In certain cases, the vesicles and prostates of castrated rats had much higher contractions and were much more excitable than the glands of normal or injected rats 3 or 5 times heavier. Moreover, the differences between the normals and the castrates were not only quantitative but also qualitative. The greater excitability of the castrate to drugs was less marked in respect to epinephrine and nicotine, to which the normal generally reacts well. With drugs of the parasympathetic group and especially with pilocarpine, the differences were striking; in our hands this alkaloid behaved as a relatively weak excitant of the normal rat genitals. The reactions exhibited by the organs were so different that the members of the staff could discern whether the donor was normal or castrate even without any knowledge of the history of the organs. The predominance of the rhythmic contractions by the organs excised from castrates was very conspicuous, as may be seen in figures 1 to 6.

We conclude, therefore, that the testicle regulates the contractility of the smooth musculature of the accessory genitalia. This regulation is made through humoral ways because, in the unilateral castrate, the contractility of the organs is normal on both sides. The behavior of the deferentia of the vasoligated rat is also normal, therefore, the surgical operation causes no interference by itself. On the other hand, the complete restoration of the motility in the castrates injected with testosterone or with its propionate, shows that this hormone is sufficient to substitute the controlling function of the testicle.

Estradiol benzoate, even in comparatively high doses, did not inhibit the contraction of the vasa deferentia, seminal vesicles, and prostates of castrated animals. In the majority of cases this substance exaggerated the excitability of the organs of the castrate. This would lead us to conclude that the follicular hormone has an action opposite to that of testosterone. However, the simultaneous administration of estradiol and testosterone to castrated animals did not antagonize or neutralize the effects of testosterone. The negative results with high doses of progesterone alone or with progesterone after estradiol benzoate simply confirmed the physiological specificities of the androgens and progesterone respectively.

It may be supposed that if the secretion of the male hormone is depressed, the contractility of the deferentia, seminal vesicles, and prostate increases. It remains to be determined if an increase in the production of estrin would give the same effects in the organism of the male; this hormone is a normal element in the male body and it is possible that it has, besides other things, the function of contributing to the regulation of that motility. In 2 normal animals injected with estradiol benzoate, the deferentia and vesicles of one behaved as those of a castrate. Although the accessory genitals were morphologically normal in this case, we think that this kind of experiment is not convincing when we consider the depressive action exerted by estrin on the testicular activity, through the depression of the anterior hypophysis.

One working hypothesis is suggested, that in the pathologic disturbances

of the contractility of the accessory genitalia (ejaculatio praecox, ejaculatio tarda, spermatorrhea, etc.) in some instances at least, the cause should be a disorder in the secretion of the sex hormone (male hormone and estrin). It would be interesting to make quantitative estimations of these hormones in the blood and urine of the patients.

Preliminary experiments with the organs in vivo on rats narcotized with urethane, binding the distal end of the deferentia and of the seminal vesicles to the myograph, suggest that, even under these conditions, the excitability in the castrate is higher; spontaneous movements which do not appear normally, appeared in the castrated animal.

The higher excitability of the organs of castrates, that we have demonstrated in vitro, seems to contradict, to a certain point, the experiment of the so-called electric ejaculation of the guinea pig, described by Batelli and Stern, and extensively studied by Moore and Gallagher (19) and Kabak (20). This is absent in castrated guinea pigs, but reappears in animals treated with testicular hormones. The contradiction is, in fact, not so evident, if we consider that in the electric ejaculation not only the excitability of the organs (deferentia, seminal vesicles, prostate) is involved, but also that of certain nervous centers and tracts; it may be that, notwithstanding the high excitability of the effector organs, the excitability of the nervous arc indispensable for the conduction of the excitation to the effector organs may be diminished. This is, of course, only a hypothesis, but it may be useful in suggesting a series of researches which may bring a solution to the problem. Moreover, we cannot yet conclude that the type of regulation of the motility of the accessory genital organs is the same in all species.

SUMMARY

A study was made of the behavior in vitro of 172 vasa deferentia, 78 seminal vesicles, and 58 prostates (coagulating gland), excised from normal, castrated, and sex-hormone injected rats. The organs were immersed in a bath of oxygenated Locke's solution, at a temperature of 37° to 38° C., and the longitudinal contractions recorded. Spontaneous contractions were not exhibited by the organs of normal rats; in those of castrates, automatism was shown in almost all cases.

Pilocarpine, acetylcholine, physostigmine, barium chloride, epinephrine, nicotine, ergotamine, and yohimbine stimulated contractions. Inhibiting or depressive effects were obtained by the application of atropine, papaverine, or spermine. After the application of ergotamine or yohimbine, the effect of epinephrine may be reversed.

Quantitative and qualitative differences in behavior were exhibited by the organs of normal and castrated rats. In the castrate, they were more reactive and predominatly rhythmic in response, especially to pilocarpine and other drugs of the parasympathomimetic group. In the normal, the reactions were predominantly tonic.

After previous storage of the isolated organs at 4° to 8° C., the reactivity to drugs and the noted differences in behavior persisted (deferentia until 13 days, vesicles until 6 days) in experiments performed at 37° to 38° C.

The treatment of castrated animals with testosterone or its propionate reestablished the normal type of behavior of the excised organs. Estradiol benzoate exaggerated the castrate type, making the organs more excitable and reactive. Progesterone alone or used after previous treatment with estradiol benzoate, at least with the doses employed, had no normalizing effect.

It is assumed that the sex hormones regulate the motility of the smooth musculature of the accessory genital organs. Inferring from the behavior of the organs in vitro, the male hormone, at least in rats, has an inhibiting action on the contractility and excitability of the vasa deferentia, seminal vesicles, and prostates. Estradiol has the opposite effect.

ADDENDUM

Since this article was written, additional experiments have been made, testing other drugs in rats (34 vasa deferentia, 27 seminal vesicles and 24 prostates). Muscarine, ephedrine and hydrastinine elicited contractions in the vas deferentia, seminal vesicles and prostates; in castrated or estradiol-treated rats, the reactions were stronger than in normal or testosterone-injected ones. Prostygmine sensitized the organs to the action of acetylcholine. Histamine gave negative results in almost all cases; in a few instances, in castrated or estradiol treated rats, slight excitation or even inhibition was observed. Other animal species have been added to the list. In rhesus monkeys (24 vasa deferentia and 19 seminal vesicles) and cats (30 vasa deferentia), the effects of castration and injection of sex hormones are substantially the same described in this paper. The differences of type of contraction (rhythmic or tonic) are not so conspicuous, but the incidence of automatism and the reactivity to drugs are higher in castrated or estradiol-injected animals. Moreover, the deferentia of cats may exhibit a very interesting variation of behavior, as regards the action of epinephrine; in castrates treated by testosterone that drug provokes contractions; in castrates receiving estradiol benzoate, the effect of epinephrine may be inhibitory. These data agree, to a certain extent, with the behavior of the cat uterus, after nervous excitation or injection of epinephrine; as Cushny, Dale, Van Dyke and Gustavson have shown, the effect may be either contraction or relaxation, depending on hormonal factors. Regarding the results referred to in this addendum see papers 21 (rats), 22 (rhesus monkeys) and 23 (cats).

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THE EFFECT OF CASTRATION ON TUBAL CONTRACTIONS OF THE RABBIT, AS DETERMINED BY THE RUBIN TEST[1]

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THE HORMONAL influence of the ovary on the morphology and functional activity of the fallopian tube has been demonstrated by many investigators. Spontaneous contraction of the tubes has been found to show cyclical variations (1, 2, 3).

The Rubin method first afforded an opportunity to study the function of the intact fallopian tube in relation to the estrous cycle (4). Rubin and Whitelaw (5) observed cyclical variations in the contractions of extirpated sow's oviducts, suspended in oxygenated Locke's solution. During estrus the contractions were rapid and rhythmical. At the diestrous period the contractions were fewer and arhythmical.

In women, with normal menses, Rubin (4, 6) found tubal contractions to be less frequent in the preovulatory phase and more frequent following ovulation. When ovarian function was diminished tubal muscle tone was lowered, and contractions were infrequent. Geist, Salmon and Mintz (7) have corroborated these findings in climacteric patients.

Moreaux (8), Westman (9) and Anopolsky (10) described morphological changes of the fallopian tube during the estrous cycle. Moreaux and Westman found the mucosa of the rabbit's tube lined with ciliated epithelium during the sexually quiescent period and after castration, while at estrus and in early gravidity it was transformed into a non-ciliated secreting structure. Anopolsky found the muscle fibers of the fallopian tube of the sow to undergo a cyclical change in length corresponding to muscle activity. The growth of the muscle fibers was correlated with the maturation of the graafian follicle and the period of rapid contractions. The fibers were smallest in the corpus luteum phase, when activity of the tube was at its lowest.

Courrier (11) found that the injection of follicular fluid into a castrated rabbit produced a reappearance of the secretory epithelium in the fallopian tube.

In this study we have noted the effect of castration on the tubal contractions of living rabbits by tubal gas insufflation, and have correlated our findings with the morphology of excised tubal specimens. We have also observed the effect of estrogenic hormones and progesterone on the contractions and morphology of the fallopian tubes of castrated rabbits.

[1] We are indebted to Dr. I. C. Rubin for the suggestion and supervision of these experiments.

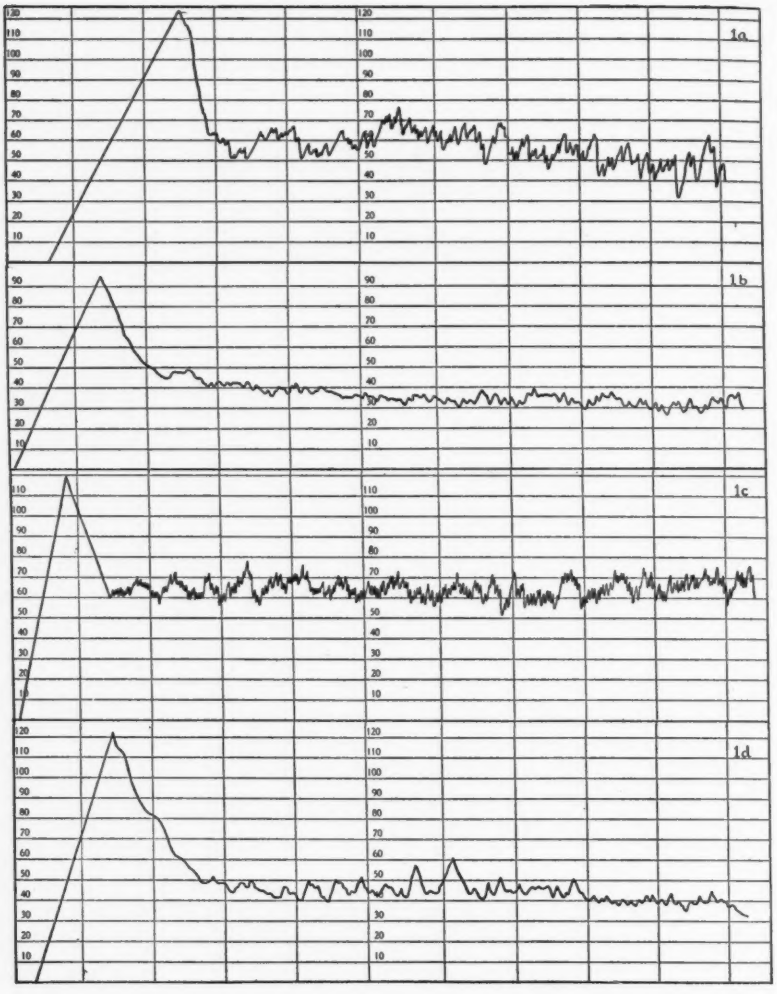

Fig. 1a. Tubal contractions of a rabbit in the anestrous stage. Fig. 1b. After surgical castration, the contractions have become less frequent and weaker. Fig. 1c. Following the injection of 800 I.U. of theelin to the castrate, the contractions were rapid and stronger. Fig. 1d. The addition of 2 mg. of proluton to the 800 I.U. of theelin injected into the castrate, resulted in a diminution in the rate of tubal contractions.

Tubal contractions were recorded on the kymograph of the Rubin apparatus during uterotubal CO_2 gas insufflation (12). The animals were anesthetized by intravenous nembutal, laparotomized, and insufflated through a cannula tied in the uterus at the uterotubal junction (13).

EXPERIMENTAL RESULTS

Comparison of tubal contractions of castrated and control rabbits. The

character of tubal contractions of 18 castrated rabbits was compared with those of an equal number of control animals. Of the latter, 11 were in the anestrous stage, and 7 were in the ovulation phase (10 hours after coitus). The average rate and height of tubal contractions were noted over corresponding periods of time and under similar conditions. In each test recordings were made for 20 to 30 minutes. The tubal contractions usually became more rhythmical and rapid after the first few minutes.

The tubal contractions of the castrated rabbits were infrequent and shallow as compared to those of the control animals. Five of the 18 castrates had no contractions for the first 5 to 10 minutes, and then an occasional weak contraction occurred. Sixteen of the 18 castrates had less than 8 tubal contractions per minute; in 9 the contraction rate was less than 5 per minute. In marked contrast, 16 of the 18 control animals had 8 or more tubal contractions per minute; in 9 the contraction rate was 10 to 15 per minute. The 7 rabbits tested during ovulation had 8 to 15 tubal contractions per minute.

The height of the tubal contractions was less than 5 mm. Hg in 11 of the 18 castrated rabbits, and in only 4 of the 18 control animals. The tubal contractions of the remaining 7 castrated and 14 control rabbits ranged in height from 5 to 10 mm. Hg (fig. 1a, b).

The effect of castration on the patency of the uterotubal junction of the rabbit has previously been described in detail (14).

Effect of estrogenic hormones and progesterone on tubal contractions of castrates. Fourteen rabbits were insufflated 1 to 2 months after surgical castration, and then tested again following intramuscular injections of estrogenic hormones. Six of these animals were subsequently injected with progesterone, and a third insufflation was performed.

Estrogenic substances were given in 4 daily doses, and insufflation was performed within 24 hours after the last injection. Four castrates were given 800 I.U., and one was given 1200 I.U. of theelin (estrone, Parke, Davis & Co.). Two were given 800 I.U., and 3 were given 1200 I.U. of progynon-B (estradiol benzoate, Schering Corporation). Three were injected with 10,000 I.U., and one with 13,000 I.U. of dimenformon benzoate (estradiol benzoate, Roche-Organon). The effect of estrogenic hormones on the tubal contractions was not found proportionate to the dosage given; smaller amounts being as effective as the larger quantities.

Progesterone was given to 6 castrates in 4 daily doses within 24 hours after the last injection of estrogenic substance. Two rabbits were given 2 mg., and one was given 3½ mg. of proluton (Schering Corporation). One rabbit was given 3½ mg., and 2 were given 4 mg. of progesterone (Roche-Organon).

The tubal contraction rate of all but one of the 14 castrates was increased after the injections of estrogenic hormones. Eight rabbits had an increase of 1 to 6 contractions per minute. Five animals had 6 to 17 additional contractions per minute. The exceptional castrate had 7 to 9 tubal contractions per minute with a height of 10 to 15 mm. Hg; following 10,000 I.U. of dimenformon benzoate, there was one strong tubal contraction per minute with a height of 60 mm. Hg.

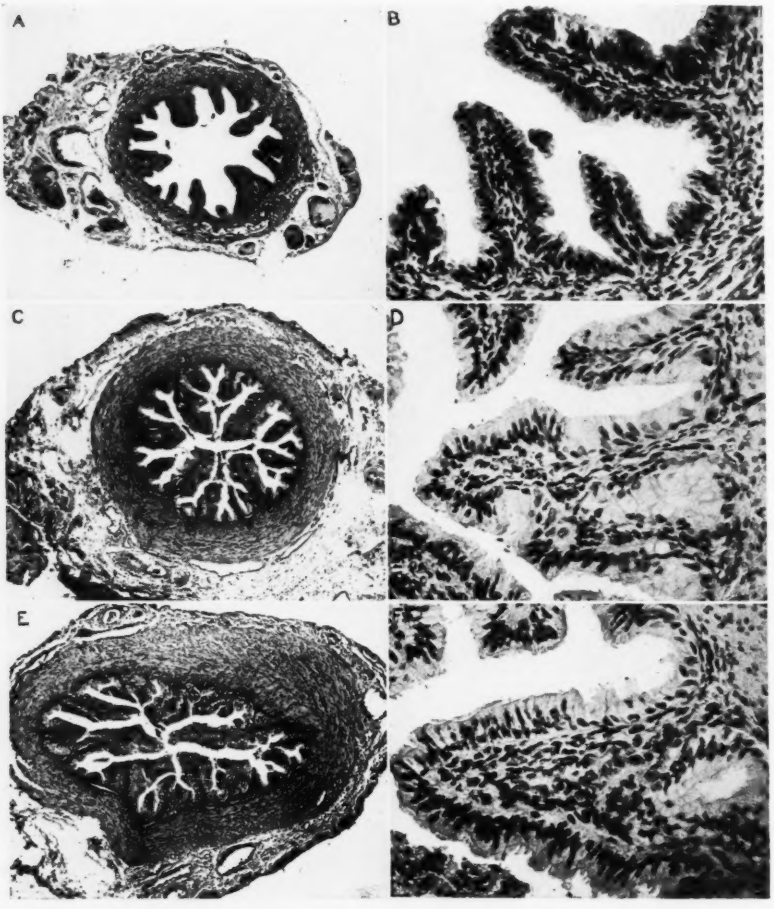

Fig. 2a. Castrate tube with atrophic mucosa and few papillary folds (×60). Fig. 2b. The mucosa of castrate tube consisting of epithelial cells of the ciliated columnar variety (×200). Fig. 2c. Castrate tube after 1000 I.U. of theelin. The papillary folds of the mucosa are more numerous and taller. The muscle layer is decidedly thicker (×60). Fig. 2d. The mucosa of castrate tube, after 1000 I.U. of theelin, consisting of high columnar epithelium with many nonciliated cells (×200). Fig. 2e. Castrate tube after 1000 I.U. of theelin and 4 mg. of progesterone (×60). Fig. 2f. The mucosa of castrate tube after 1000 I.U. of theelin and 4 mg. of progesterone (×200).

The tubal contractions of 12 of the 14 rabbits were 5 to 35 mm. Hg higher after the injections of estrogenic hormones. In 2 instances there was no noticeable change.

Five castrates had no contractions for the first 5 to 10 minutes of the tubal insufflations, and then small contractions were described on the kymograph. The administration of estrogenic substances to these animals was followed by spontaneous tubal contractions from the beginning of the tests (fig. 1b, c).

The tubal contraction rate of 3 rabbits was less after the addition of

progesterone than when estrogenic hormones were given alone; in 3 instances there was no noticeable change. The height of contractions appeared to be unaffected by the progesterone (fig. 1d).

Effect of estrogenic hormones and progesterone on tubal morphology of castrates. The fallopian tubes of the castrates were atrophic. The mucosa contained few papillary folds. The epithelial cells were mostly of the ciliated columnar variety with scant cytoplasm, and with deeply staining basilar nuclei. The muscular layer was thin (fig. 2a, b).

After the castrated rabbits were injected with estrogenic hormones, the papillary folds of the mucosa were more numerous and taller, almost filling the lumen. The epithelial cells were higher, mainly non-ciliated, and contained more cytoplasm. The nuclei were more vesicular. The muscular layer was decidedly thicker (fig. 2c, d).

After the castrated rabbits were injected with estrogenic hormones and progesterone, the microscopic picture was similar to that obtained with estrogenic substances alone (fig. 2e, f).

SUMMARY

The character of tubal contractions of 18 living castrated rabbits was compared with that of an equal number of control animals, by uterotubal CO_2 gas insufflation. After castration, the contractions were less frequent and weaker.

The administration of estrogenic hormones to 14 castrated rabbits resulted in an increase in the rate and height of tubal contractions. The addition of progesterone to 6 of these animals, sensitized with estrogenic substances, resulted in a diminution of the contraction rate in 3 instances and no variation in the remaining 3.

After castration, the tubal mucosa of rabbits became atrophic and the muscular layer thin. Following injections of estrogenic hormones and progesterone, the tubal mucosa of castrates became hypertrophied and the muscular layer thicker.

These findings lend further support to the vast evidence already accumulated of the hormonal influence of the ovaries upon the contractions of the fallopian tubes. We have again demonstrated the value of uterotubal gas insufflation as an indicator of ovarian function.

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PHYSIOLOGICAL STUDIES IN INSULIN TREATMENT OF ACUTE SCHIZOPHRENIA

METHODS[1]

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BETWEEN SEPTEMBER, 1937, and September, 1938, a project on insulin therapy in schizophrenia was conducted on the research wards of the Worcester State Hospital, the object of which was to throw some light on the psychological and physiological factors involved, especially in remissions. In the following papers individual research workers are reporting on some of their physiological findings. Papers on the psychiatric and psychological aspects of this project will be published separately.

In order to avoid the repetition of descriptions of the patient sample and of experimental conditions, these points will be dealt with briefly in this introductory paper. The main object here, however, is a discussion of the statistical methods, and of the interpretational properties of the statistics used in the analysis of the data.

Description of Sample and of Procedures

The sample under consideration consisted of 22 male schizophrenic patients free of all complicating diseases. With one exception they were all first admissions. They were in the age range of 15 to 44 years with only 3 patients under 20 years. The period of hospitalization at the time insulin treatment started ranged from a few days to a maximum of 18 months. Only 3 patients had been in the hospital more than 6 months at the start of insulin treatment. Further details relative to age and hospital stay are given in table 1.

TABLE 1. RANGE AND MEANS OF AGE AND DURATION OF HOSPITALIZATION OF INSULIN PATIENTS

	No.	Age, years			Hospitalization, months		
		Min.	Max.	Mean	Min.	Max.	Mean
All insulin pts.	22	15	44	27.0	0.2	18.0	3.0
'Recovered' pts.	10	17	44	26.8	0.2	10.0	2.2
'Not-recovered' pts.	12	15	40	27.2	0.2	18.0	3.6

[1] We wish to acknowledge our indebtedness to the Eli Lilly Company for supplying us with the Iletin Insulin used for this study.

The condition of patients after stopping treatment was judged by 4 psychiatrists. There were only minor differences in judgment. Ten of the 22 patients went into remission. These 10 patients who comprise both the full remissions and the social recoveries will be referred to for convenience in the following papers as the 'recovered' patients, while the remaining 12 patients will be referred to as the 'not-recovered' patients. No implication is intended that any patient was genuinely cured.

The 'recovered' patients were all well enough to be sent home, but differed to some extent in the depth of the remission. Among the 'not-recovered' patients there were some who had shown improvement, but not sufficient to warrant returning them to their homes. Occasionally reference will be made to normal controls. These did not receive insulin, but form simply a basis for comparison of the pre-medicational values of patients. The values for normal controls were obtained previously at this institution on healthy males, ranging in age from 20 to 45 years, with a mean of 26 years (1).

Data relative to insulin dosages and number of treatments are given in table 2. As other studies have also shown (2), the number of treatments was somewhat higher for the 'not-recovered' patients. This is a natural consequence of trying to give the treatment a full chance. In all other respects, the differences between the various dosage means of the 'recovered' and 'not-recovered' patients are not beyond random variation.

TABLE 2. RANGES AND MEANS OF NUMBER OF TREATMENTS AND OF DOSAGE OF INSULIN PATIENTS

	No.	No. treatments			Total dosage, U			Average dosage, U			Average dosage per body wt., U		
		Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
All insulin patients	22	22	86	44.5	1200	5860	2947	44.3	111.6	66.8	0.72	2.05	1.09
'Recovered' patients	10	22	53	40.5	1260	5470	2948	50.5	111.6	70.4	0.79	2.05	1.23
'Not-recovered patients	12	25	86	47.9	1200	5860	2946	44.3	90.0	63.7	0.72	1.84	0.97

Since pre-medicational and post-medicational values of various blood constituents are to be compared, it is of importance to note that the pre-medicational mean hematocrit value was 44.4 and the post-medicational mean was 44.2. By 'insulin treatment' the complete course of n insulin administrations is meant, not a single administration. In single administrations the hematocrit takes an entirely different course. It rises from an initial mean value of 44.0 to 46.8 during coma, and falls back to the initial value after administration of sugar.

Except for blood pressure, all tests were carried out in a state of post-absorption, that is, under basal conditions. All variables under consideration were tested a fixed number of times, and at strictly fixed intervals during the 2 or 3 weeks preceding insulin administration, and with the same number of repetitions and at the same intervals for the 2 or 3 weeks after completion of therapy. The stipulation of an equal number of repetitions for

all individuals is an essential one for the determination of trends, while the stipulation of entirely fixed intervals is essential for the determination of intra-individual variation (3). Single observations will be referred to as 'readings.' In referring to the average of the 1st reading is meant the average of the 1st readings of all the 'recovered' or of all the 'not-recovered' patients. Any systematic shift in the means of readings will be referred to as a 'trend.' The aggregate of the readings before insulin therapy will be referred to as the 'premedication' study, and the aggregate of the readings after the termination of therapy will be referred to as the 'post-medication' study.

The testing schedule was arranged so that while one patient may have had his post-medication study another subject was entering on the pre-medication study. This staggering of the testing program contributes largely to the elimination of possibly confusing seasonal variation and other artefacts of experimentation which might obscure the effect of insulin on the variables under investigation.

Statistical Considerations

As mentioned before, the sample consists of 22 patients. When it is subdivided into 10 'recovered' and 12 'not-recovered' patients, the samples may seem too small for profitable analysis. Large random samples permit of drawing conclusions relative to the parameters of the populations from which the samples have been drawn. Describing these populations from small random samples is entirely illusory. Two estimates derived from two small samples, however, may be used to test the hypothesis that the two samples have been drawn randomly from the same population. Such a test is expressed in terms of probability, and a given probability value derived from 10 or 15 observations has the same meaning and significance as the same probability value derived from tens of thousands of observations. Differences between means and between measures of variations may be tested on small samples. There are, however, two important limitations. One is that much greater reliability attaches to the significance than to the non-significance of differences. The other limitation is that one can merely assert that a significant difference obtains, but not how great the difference actually may be. All this is valid only in the case of random sampling, which is the sine qua non of all statistical conclusions.

The data were arranged first according to whether they belonged to 'recovered' or 'not-recovered' patients, and whether they pertained to the pre-medication or post-medication study. Thus we obtained 4 main sets of data for each variable. Complete analyses of variance were carried out on each set separately, as well as on various combinations of sets. In order not to hamper the discussion with technicalities the significance of the differences between means, originally determined in the analysis of variance will be given in terms of the more familiar t test (4). Of the results of the analysis of variance only estimates of the variation within the individual will be utilized, and these will be given in terms of the intra-individual standard deviation. The significance of the differences between these standard devia-

tions is determined by forming the ratios of their squares, that is, of the variances. Probability values attaching to these ratios are conveniently tabulated (5). Probabilities between .05 and .01 are regarded as indicating probable significance, and probabilities of less than .01 as indicating high significance.

The group variation, that is, the inter-individual variation, will not be discussed, nor shall we touch on measurements of the differential response. 'Reading' trends will be mentioned superficially only. A more technical paper will deal with these complex types of variation.

The reason for centering the discussion on the intra-individual variation is that in schizophrenic individuals this has been found to be far in excess of that of normal control subjects (6). It is, therefore, of great interest to investigate whether or not insulin treatment has any effect on the intra-individual variation, and whether or not there is any concomitance between changes in the magnitude of this variation and changes of the clinical status of the patients. Intra-individual variation significantly in excess of the normal is interpreted as instability of the function in question. Decreases or increases of this function following insulin treatment are interpreted in terms of the stability of the function.

For all variables the changes between the levels of the pre-medication and the post-medication study were correlated with the insulin dosage. This was done in terms of the 'total dosage,' the 'average dosage' and the 'average dosage per kilogram body weight,' as well as in terms of the number of treatments. Whenever it is stated that no correlation was found between the change in a certain variable and the magnitude of the insulin dosage, it must always be understood that this refers to all the alternatives mentioned above. In some instances, statistical procedures were used which do not appear in any of the other papers, and these, as far as they require, are explained in footnotes.

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PHYSIOLOGICAL STUDIES IN INSULIN TREATMENT OF ACUTE SCHIZOPHRENIA

PULSE RATE AND BLOOD PRESSURE

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THE PULSE RATE and blood pressure were investigated as part of an extensive study of the effects of insulin treatment of schizophrenia. In view of earlier indications of an increased sympathetic activity (1, 2) following prolonged treatment, it was considered that investigation of these two criteria might yield valuable information.

Pulse Rate

Basal pulse rates were observed on each of 22 patients suffering from schizophrenia of recent onset. The pulse rates were recorded on 4 occasions in the 2 weeks preceding insulin treatment and on 4 occasions in the 2 weeks following treatment. Four observations of the basal pulse in normal subjects were also recorded over a 2-week period. The mean pulse rates and the intra-individual standard deviations are given in table 1.

TABLE 1. MEANS AND INTRA-INDIVIDUAL STANDARD DEVIATIONS OF THE PULSE RATE (BEATS PER MINUTE)

	23 Normal controls	Patients			
		10 'Recovered'		12 'Not-recovered'	
		Before medication	After medication	Before medication	After medication
Mean	62.2	61.7	67.6	63.7	63.2
Intra-individual standard deviation	3.9	6.0	4.1	7.4	7.1
		Significance of difference between means	$t=2.39$ $P_t=0.04$	Significance of difference between means	$t=0.28$ $P_t=.77$

Both groups of patients had initially approximately the same mean pulse rate as the normal controls, but were markedly less stable in this function as indicated by the high intra-individual standard deviations. The 2 groups, however, did not differ from each other initially in mean level or intra-individual variation.

Following insulin treatment the 'recovered' patients increased signi-

ficantly in their mean pulse rate level, while the 'not-recovered' patients showed no change. This is of great interest, since many variables involved in the insulin project showed significant changes for the 'not-recovered' as well as for the 'recovered' patients. The fact that only the 'recovered' patients showed an increase in the average level of the pulse rate indicates that the action upon the mechanism controlling the circulatory functions is an integral part of the therapeutic response to insulin. This will be borne out later by the fact that in blood pressure also only the 'recovered' patients showed significant increases.

While the 'not-recovered' patients maintained their supra-normal intra-individual variation after insulin treatment, the variation in the 'recovered' patients dropped to the normal level. That is, insulin not only raised the average level of the pulse rate but also stabilized this function in the individual patients.

A correlation between the magnitude of the change in pulse rate and the magnitude of the average insulin dosage showed that the changes did not depend upon dosage. An insignificant correlation coefficient of .25 was found.

Blood Pressure

Observations of the blood pressure of our patients were made on 20 occasions before the commencement and on 20 occasions after the termination of insulin therapy. The observations were made at 8:30 A.M., 12:00, 1:30, 3:30 and 5:00 P.M. of 4 different days. This procedure was selected in order to obtain within a short period a sufficiently large number of readings to allow statistical analysis both of the group and of the individual findings. Means of the systolic blood pressure are shown in table 2.

TABLE 2. MEANS OF SYSTOLIC BLOOD PRESSURE (MM. HG) AND CRITERIA OF SIGNIFICANCE OF DIFFERENCE OF MEANS

	26 Normal controls	Patients			
		10 'Recovered'		11 'Not-recovered'	
		Before medication	After medication	Before medication	After medication
Mean	115.3	107.6	115.9	114.9	114.2
		Significance of difference between means	$t=3.11$ $P_t=0.01$	Significance of difference between means	$t=0.35$ $P_t=0.70$

The 'recovered' patients were initially significantly below the mean of the normal controls, while the 'not-recovered' patients had practically a normal mean value. The difference between the 'recovered' and 'not-recovered' patients becomes even greater if one takes into account the fact that both types of patients habituated (3) in the course of the 4 days of observation, and that on the fourth day the blood pressure of the 'recovered' patients averaged 104.8 mm. Hg while that of the 'not-recovered' patients averaged 112.2 mm. Hg. Due to the smallness of the two samples, statistical

significance of the difference cannot be established, but the indications are that on increasing the samples a reliable difference may be found. It seems then that while on the whole the average systolic blood pressure of patients in the acute stage of schizophrenia is not below normal, there is a group in which the blood pressure is tending towards hypotension, and it is this group which is likely to respond favorably to insulin. It does not seem unreasonable to infer from this that a lowered sympathetic activity is a favorable ground for insulin therapy in schizophrenia.

The group of 'recovered' patients were not only differentiated from the 'not-recovered' patients by an initially lowered systolic mean but also by their response to insulin. Their systolic blood pressure increased after treatment significantly from 107.6 to 115.9 mm. Hg while the 'not-recovered' patients did not change in their mean level. The intra-individual variation of the blood pressure showed no interesting features.

The mean of the diastolic blood pressure for 'recovered' as well as for 'not-recovered' patients was initially close to the normal mean of 67.3 mm. Hg. While there was some rise in the course of insulin treatment in the 'recovered' patients, this rise was not significant.

The Systolic-Diastolic Relation

The difference between the systolic and diastolic blood pressure, commonly termed the pulse pressure, is usually treated as a separate variable. It seems to us more profitable to talk in terms of the degree of simultaneous variation or covariation of these two phases of blood pressure. This covariation is measured by the correlation coefficient designated as r.

It has been suggested that the correlation coefficient between the systolic and diastolic blood pressure is a relevant index of sympathetic activity (3, 5). It can be demonstrated that in a rigid system of tubes there is an absolutely constant relationship between the pressure at the upstroke and the pressure at the downstroke of the pump. In other words, the correlation in such a system is a perfect positive one, and r is unity. If the diameters of the tubes are varied irregularly during the operation of the pump, the relationship between the two phases of pressure will be loosened and will become more and more inconsistent as the frequency and irregularity of changes in diameter increase. The circulatory system represents a structure of tubes in which irregular changes in diameter are brought about through impulses emanating largely from the sympathetic nervous system. If the sympathetic were under-active, one would expect a high correlation between the systolic and the diastolic blood pressure, and conversely a low correlation or lack of correlation may be expected with over-activity of the sympathetic. It must be pointed out, however, that a high correlation may come about in spite of normal activity of the sympathetic if the blood vessels themselves are relatively rigid, and thus unresponsive (arteriosclerosis). From investigations of the correlation between the heart rate response to ice water and the systolic-diastolic r (6), it seems probable that while the ice-water test records the potential responsiveness of the adrenal-sympathetic system, the systolic-

diastolic r records the degree to which that system responds to the business of living.

Previous investigations carried out at this institution on large samples have yielded for normal controls an average correlation coefficient of .43 between the systolic and diastolic blood pressure. For schizophrenic patients the average correlation coefficient was .63. This was interpreted as an indication of sympathetic sluggishness in schizophrenia. Individuals showed marked differences in their systolic-diastolic correlation. Occasionally negative correlation coefficients were found but these were of such small magnitude that they could not be regarded as anything but random deviations from a zero correlation.

It must be emphasized that the average correlation coefficients given above pertain to subjects in the basal state. In the present investigation blood pressure observations were made in the non-basal state in order to facilitate the collection of sufficient observations on each individual in a short space of time. Now, since in the non-basal state nervous activity is at a higher level, a lower systolic-diastolic correlation must be expected than in the basal state. Normal control values are available for the latter only, but let us surmise that the normal average for the non-basal state would be close to .30. The average pre-medicational correlation coefficient for the patients who subsequently recovered was .45 and for the patients who did not recover it was .48.[1] These coefficients must be regarded as fairly high for non-basal conditions. Without any further statistical test it is evident that the latter two coefficients are not significantly different from each other. After termination of insulin treatment, the 'recovered' patients had an average correlation coefficient of .29 and the 'not-recovered' patients averaged .56. This difference is highly significant.[2]

The changes in the systolic-diastolic correlation indicate that in the 'recovered' patients an activation of the sympathetic nervous system was brought about, while in the 'not-recovered' patients sympathetic deterioration may have occurred. It is not implied that this deterioration in the 'not-recovered' patients is due to insulin. The implication is rather that insulin had no effect on these patients, and the schizophrenic process progressed from the acute to the sub-acute stage.

DISCUSSION

The data on the pulse rate and blood pressure are interesting from two points of view. First, the light which they throw upon the pre-medication differences which exist between patients who respond well to insulin and those who do not, and on the differences which exist between both groups

[1] The averaging of correlation coefficients was performed by transforming each individual correlation coefficient into the corresponding 'z' quantity. $z = \tanh^{-1} r = \frac{1}{2}[\log_e(1+r) - \log_e(1-r)]$. The z's were averaged, and the average z was retransformed into r. The standard error of z is $1/\sqrt{n-3}$. It be noted that an r averaged in this way has the same significance as an r emanating from the combined sets.

[2] $r = .29$, $z = .2937$; $r = .56$, $z = .6356$. Difference of z's $= .3419$, and since the former z is estimated from 10 times $(20-3)$ and the latter z from 12 times $(20-3)$ readings, the standard error of the difference is $\pm .104$. The difference exceeds its standard error 3.29 times and is highly significant.

and normal subjects. Second, they are of interest because of the information which they afford as to what changes seem to be associated with improvement. The outstanding difference shown by the pre-medication pulse rate was the greater 'within-individual' variability. Theoretically it is possible to think of this increase in variability as arising either in consequence of acceleration due to an over-active adrenal-sympathetic or as arising from irregular control due to a defective adrenal-sympathetic system. In view of the fact that the average pulse rate did not differ significantly from the normal, the latter hypothesis seems the more tenable. This viewpoint is lent further support by the blood pressure findings. It was found further that in the patients who 'recovered,' the average pulse rose significantly and failed to do so in those who did 'not recover.' Similarly the variability fell to normal limits in the 'recovered' patients and remained much the same in the other patients.

In the blood pressure investigations the outstanding facts were that the pre-medication average systolic reading was found to be significantly lower in the 'recovered' group than in normals, whereas it was not in the 'not-recovered' group; following on treatment it rose to normal limits, while there was no change in the 'not-recovered' group.

SUMMARY

Insulin treatment was carried out on 22 patients suffering from recently developed schizophrenia. Pulse rates and blood pressure readings were recorded before and after treatment.

The 'recovered' patients showed before treatment greater pulse variability than normal, but less than the 'not-recovered' patients. After treatment the variability of the 'recovered' patients returned to normal limits, while the 'not-recovered' showed little change.

The average pulse rate of the 'recovered' patients increased significantly to beyond the normal level, and that of the 'not-recovered' patients remained at normal level.

Before the treatment the 'recovered' group showed a tendency towards hypotension. The systolic-diastolic correlation was high in both groups. After treatment the 'recovered' group became approximately normal in all recorded aspects of the blood pressure, while little or no change was noted in the 'not-recovered' group.

Evidence is presented which suggests that prior to treatment there is some degree of adrenal-sympathetic impairment and that this is largely overcome by treatment in the 'recovered' patients and relatively unaffected in the 'not-recovered' patients.

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PHYSIOLOGICAL STUDIES IN INSULIN TREATMENT OF ACUTE SCHIZOPHRENIA

THE SERUM LIPIDS

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THE INCLUSION of a study of the serum lipids in a project on insulin therapy of schizophrenic patients does not require elaborate justification. The variation of these blood constituents in the insulin treatment of diabetes, as well as the more recently suggested rôle of the adrenal cortical hormones and the sympathetic nervous system in the regulation of body fats, are sufficient reasons for the investigation (1). An additional justification is the variously reported, although almost as frequently negated, deficiency of serum lipids in schizophrenic subjects (2).

In an earlier study of the effect of insulin treatment on whole blood lipids (4), it was found that a mild lipemia occurred during the early weeks of treatment in 14 of 16 cases studied. In 5 of these patients, showing remission, the whole blood phospholipid, total cholesterol and total lipid remained elevated after stopping treatment, while in those patients who did not show remission the lipids dropped towards the initial level. We undertook the present investigation in order to extend our observations to a larger number of patients; to study the serum lipids which reflect changes in fat metabolism more clearly than the whole blood; and to study the relation of free cholesterol to cholesterol ester.

METHODS

The total lipids, phospholipids, total cholesterol, free cholesterol and cholesterol ester were determined once in each of the 2 weeks preceding treatment, and once in each of the 2 weeks following the end of treatment. Blood from the antecubital vein was taken in the post-absorptive state and 5 cc. of serum was extracted according to Bloor's technique. Aliquots of the alcohol-ether extract were analyzed in duplicate for phospholipid and total lipid by Bloor's methods (4) and free and total cholesterol by Turner's modification of Okey's method (5). In this modification, washing in centrifuge tubes is substituted for the filtration procedure. All other parts of the procedure were identical with the Okey method. Cholesterol ester was determined by difference.

RESULTS AND DISCUSSION

The means of phospholipids, cholesterol ester and free cholesterol are

TABLE 1. MEANS OF SERUM LIPIDS (MG./100 CC.) AND CRITERIA OF SIGNIFICANCE OF DIFFERENCE OF MEANS

	21 Normal controls	10 'Recovered' pts.		12 'Not-recovered' pts.	
		Pre-medication	Post-medication	Pre-medication	Post-medication
Phospholipids	214	184	203	178	209
Cholesterol ester	134	111	131	115	130
Free cholesterol	52	48	51	53	48

SIGNIFICANCE OF DIFFERENCES OF MEANS IN TERMS OF THE *t* CRITERION

	Phospholipid		Cholesterol ester	
	t	P	*t*	P
'Recovered' pts.				
Pre-medication vs. normal controls	2.33	.03	2.64	<.02>.01
'Not-recovered' pts.				
Pre-medication vs. normal controls	3.12	<.01	2.90	<.01
'Recovered' pts.				
Pre-medication vs. post-medication	2.44	.04	3.63	<.01
'Not-recovered' pts.				
Pre-medication vs. post-medication	4.72	<.01	2.24	.05

(*t* criteria for free cholesterol are not given since the differences are obviously not significant.)

given in table 1. Supplementary information may be found in figures 1 and 2 which present all observations in the form of dot distributions.

The 'recovered' and 'not-recovered' patients did not differ from each other initially in the mean levels of their serum lipids. Both of these groups, however, had initially significantly lower means than normal controls for phospholipids as well as for cholesterol ester. In the case of free cholesterol, however, the differences of the means were not beyond random variation.

The phospholipids and cholesterol ester both showed significant increases in their mean levels after insulin therapy in the group of the 'recovered' as well as in the group of the 'not-recovered' patients. The magnitude of the increase in the mean levels of these two variables was approximately the same in both groups of patients. There were no systematic changes in the mean levels of free cholesterol.

Changes in the total lipids were proportionate to the changes of the separate constituents discussed here.

While changes in the mean levels did not differentiate between 'recovered' and 'not-recovered' patients, the behavior of the intra-individual variation distinguished the two patient groups rather markedly. The intra-individual standard deviations are given in table 2.

TABLE 2. ESTIMATES OF INTRA-INDIVIDUAL STANDARD DEVIATIONS OF SERUM LIPIDS (MG./100 CC.)

	21 Normal controls	10 'Recovered' patients		12 'Not-recovered' patients	
		Pre-medication study	Post-medication study	Pre-medication study	Post-medication study
Phospholipids	7.1	9.4	6.6	11.1	28.3
Cholesterol ester	5.7	10.3	7.4	8.1	12.7
Free cholesterol	2.6	3.2	6.7	4.4	6.2

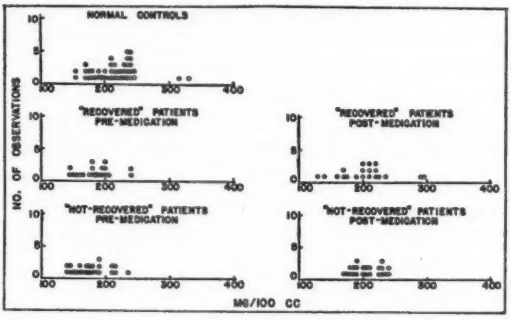

Fig. 1. FREQUENCY DISTRIBUTIONS OF SERUM PHOSPHOLIPIDS.

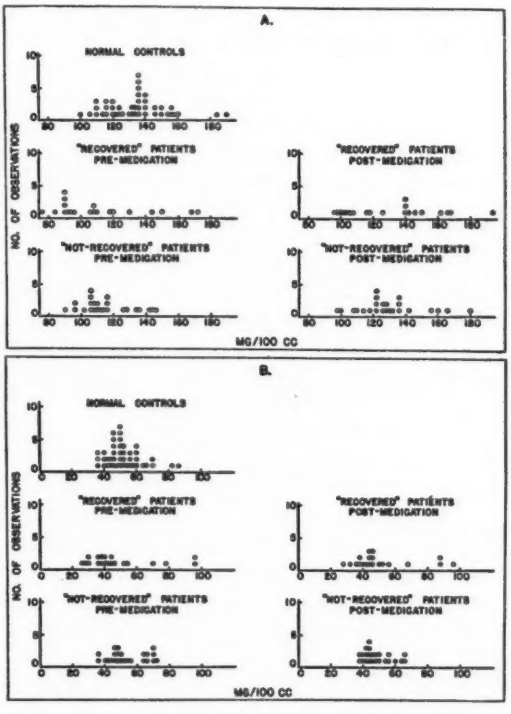

Fig. 2. *A*, FREQUENCY DISTRIBUTIONS OF SERUM CHOLESTEROL ESTER. *B*, FREQUENCY DISTRIBUTIONS OF SERUM FREE CHOLESTEROL.

Two features stand out sharply in this table. The first is that the intra-individual standard deviations in both patient groups were initially higher than in normal controls for all the variables listed here. Individually only some of these differences are statistically significant, but the difference is so

consistent from variable to variable that we may attach significance to all of the differences. These patients are, therefore, characterized by great instability of the controlling mechanism of the serum lipids.

The other feature is that with the exception of free cholesterol the intra-individual variation decreased significantly in the 'recovered' patients and increased significantly in the 'not-recovered' patients after insulin treatment. The essential difference in the behavior of the two groups is thus that the controlling mechanism of the serum lipids became stabilized in the 'recovered' patients, while in the 'not-recovered' patients instability of the controlling mechanism became even more marked than it was initially.

The classical studies of Sperry (6) have shown that the ratio of cholesterol ester to free cholesterol is highly consistent within the individual as well as among individuals. The variation of the two components must show, therefore, high concomitance; in other words, a controlling mechanism must regulate the covariation of the two cholesterol fractions. There are some indications that this regulating mechanism may be of enzymatic nature, and probably at least indirectly related to adrenal functions.

The most straight-forward measurement of the consistency of the relation of the two cholesterols is in terms of the intra-individual variation of the ratio of cholesterol ester to free cholesterol. Computations on Sperry's data yielded an intra-individual standard deviation of 0.17. On our own normal controls an intra-individual standard deviation of 0.14 was obtained; these two results do not differ by more than random variation. This measure of consistency will serve as a basis for the determination of the consistency in our two types of patients before and after insulin medication.

The consistency of the ratio was poor initially in the subsequently recovered patients. Their intra-individual standard deviation of 0.30 was significantly greater than that of normal controls, as well as that of the 'not-recovered' patients (0.17). These two types of patients thus differed initially in respect to the efficiency of the synchronizing mechanism of the two cholesterol components. The anomaly attaches to the patients who subsequently recovered, and it is suggested that this anomaly, together with others described in the preceding papers, form a complex which furnishes a suitable ground for the favorable action of insulin. After cessation of insulin treatment the consistency of the ratio of the two cholesterols became normalized in the 'recovered' patients (intra-individual standard deviation, 0.17), but became disrupted in the 'not-recovered' patients (intra-individual standard deviation, 0.33).

The fact that following insulin treatment the variation became more stable in the 'recovered' patients but more unstable in the 'not-recovered' patients has been mentioned repeatedly above. There is a feature common to all the lipids studied which complements this finding and sheds additional light on the differences in response between the two types of patients. The gains in average level were maintained by the 'recovered' patients in the 2 weeks following the termination of treatment but were not maintained by the 'not-recovered' patients, as can be seen from the tabulated weekly means.

While only the trend for cholesterol ester is statistically significant, the other trends may be regarded as significant by the criterion of consistency. It should not be assumed that the increased post-medicational intra-individual variances of the 'not-recovered' patients are due to this downward trend. The trend is eliminated in the estimates of the 'net' intra-individual variances which are given when 'reading trends' are significant.

Gellhorn (7) considered that insulin hypoglycemia produced an anoxemia of the brain followed by a stimulation of the sympathetico-adrenal system. Many somatic symptoms of schizophrenia resemble those of adrenal insufficiency, particularly the instability of the sympathetic system with its attending symptomatology. It is conceivable that insulin, by virtue of its

TABLE 3. MEAN LIPID LEVELS OF 'RECOVERED' AND 'NOT-RECOVERED' PATIENTS IN THE 2 WEEKS FOLLOWING INSULIN MEDICATION

		Post-medication study	
		Mean of 1st week	Mean of 2nd week
Phospholipid	'Recovered' patients	201	206
	'Not-recovered' patients	214	205
Free choles	'Recovered' patients	49.7	51.4
	'Not-recovered' patients	49.0	47.8
Choles. ester	'Recovered' patients	131	131
	'Not-recovered' patients	135	123

action in stimulating the sympathetico-adrenal system causes improvement in the organic symptoms of the patient. Thus the low blood lipid levels in the acute schizophrenic patient may be correlated with the hypometabolism and underactivity of the sympathetico-adrenal system and the rise in lipids during insulin treatment may be correlated with the stimulation of the sympathetico-adrenal system and improved metabolism. Why some patients improve mentally under the treatment and are able to maintain blood lipid levels, metabolic activity and blood pressure at their optimum while others do not is entirely conjectural. It may be that those patients who have the most unstable sympathetico-adrenal system are most susceptible to improvement under treatment.

A possible correlation between the nutritional status and the serum lipids would make one inclined to think that the increase in the lipids following insulin medication may be contingent on the weight gains usually occurring in such treatment.

It must be considered, however, that the correlation between body weight and serum lipids obtains only in the extremes. Low lipids are found in emaciation, and high lipids in obesity, but between these extremes there is no covariation between these two variables (8). Although about half our patients were underweight, they were by no means emaciated. The pre-medicational lipid values were plotted against the pre-medicational nutritional indices, and showed only the slightest trace of a trend at the lower range end of the nutritional indices. The changes in the lipid values were plotted against the changes in weight, but the highest correlation obtained was not more than .29.

The pre-medicational level of the lipid values and the magnitude of the insulin dosage also did not account for the magnitude of increases in the various lipids.

SUMMARY

Twenty-two patients in the acute stage of schizophrenia were tested for serum lipids (phospholipids, total cholesterol, free cholesterol, cholesterol ester and total lipids) in the 2 weeks preceding and in the 2 weeks following insulin treatment.

All the mean lipid values, except free cholesterol, were initially significantly lower than the values obtained in normal controls.

Following medication, the levels of all the lipids, except free cholesterol, were increased significantly, reaching the normal level in the 'recovered' as well as in the 'not-recovered' patients.

Differences between these two types of patients were found in the behavior of the intra-individual variation. In the 'recovered' patients, the intra-individual variation was greatly lowered after treatment, indicating that the lipid regulating mechanisms became stabilized. In the 'not-recovered' patients, however, the chemical action of insulin in raising the lipid levels was achieved only at the expense of increased instability of the regulating mechanism.

The increase in lipid values was maintained by the 'recovered' patients durings the 2 weeks following termination of insulin treatment, while the 'not-recovered' patients tended to drop back towards the initial level.

Initially, the ratio of cholesterol ester to free cholesterol showed great inconsistency in the subsequently recovered patients, but normal consistency in the 'not-recovered' patients. This initial anomaly in the subsequently recovered patients forms a syndrome with other anomalies, such as hypotension and under-nutrition, and it is suggested that this syndrome forms a suitable basis for the favorable action of insulin.

The cholesterol ester—free cholesterol ratio became stabilized in the 'recovered' patients but instability was increased in the 'not-recovered' patients following insulin treatment.

Increases in the lipid levels were independent from changes in weight, from the initial lipid levels, and from the magnitude of the insulin dosage.

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THE EFFECT OF ESTROGENIC HORMONE ON VESICAL MUSCLE

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THE AMOUNT of fluid which the urinary bladder of a deeply anesthetized animal will hold before overflow occurs has been found to be remarkably constant for the normal individual. Under narcosis reflex contraction is largely inhibited, and the bladder behaves as an inert container which overflows passively when overfilled (1). We speak of the maximal vesical capacity or vesical volume. Certain disturbances such as section of the nerves will change this factor which we have used as an indicator in these experiments. Such variations must indicate modifications in the tone and contraction of the vesical muscle.

In a previous communication, we have shown that pregnancy in the rabbit is characterized by a definite increase in the maximal vesical capacity (2). Often the vesical volume during pregnancy is two or three times that in the non-pregnant animal. Following the termination of pregnancy, the maximal vesical volume is greater than at any previous period and the bladder does not return to normal size until three or four weeks after delivery. The relative diminution in capacity before delivery and the increase following delivery are thought to be the result of pressure from the enlarged uterus in the former, and removal of this pressure in the latter instance.

We have shown further that a similar increase in vesical capacity occurs following ovulation in the normal rabbit in approximately 50 per cent of the cases. Ovulation was produced by intravenous administration of the anterior pituitary-like sex hormone (antuitrin-S). Since no increase in bladder capacity occurred in a single instance in a control series of castrated rabbits following the administration of antuitrin-S in the same doses, it was felt that the changes in vesical capacity observed in the series of normal rabbits was significant.

It has been shown by Woolsey and Brooks (3) that micturition volume diminishes markedly during estrus or while the animal, female or male, is under the influence of the estrogenic hormone. Their studies were made by placing cats for long periods of time in metabolism cages equipped to record continuously the volume and time of micturition.

The observations of Woolsey and Brooks together with our own observations convinced us that in addition to its neurological control, the behavior of the vesical muscle is influenced to some extent by the hormones present in the blood of the animal. The estrogenic hormone and progesterone appear to influence the vesical muscle in a way similar to that in which they influence

the uterine muscle. The object of the present study is to determine the effect of the estrogenic hormone on bladder muscle.

METHODS

For this study castrated cats were used rather than rabbits, since catheterization of the urethra in the cat is easier than in the rabbit. The castrated animals were given several weeks to recover from the operation. They were always anesthetized with pentobarbital injected intraperitoneally at the time a reading was made. A glass catheter was inserted into the bladder and

TABLE 1. EFFECT OF ESTROGENIC HORMONE ON VESICAL CAPACITY

Cat No.	1st control	7 days later	7 days later				7 days later	7 days later	7 days later
	First Series								
1		41	35		20		35	30	
3	30	50	30		23.5		28.5	44	51.5
6		25	25		20		20	24.5	
9	35	35	40		55		20	25	
11	55	35	45		20		75	85	60
12		30	40		35		33.5		
13		50	55	*Estrogenic hormone started*	25	*Estrogenic hormone stopped*	48.5	48	
14	43.5	37	44.5		35		48		
15	35	25	41		35		35		
16		40	42		26		28.5	80	
18		42	45		29		59	50	
	Second Series								
1		35	40		20		36.5	27	33
3		35	30		20		55	45	
6		17	23		13.5		15	28	24
9		40	39		30		20	90	21
11		34	30		24		75	65	70
13		30	38.5		20		19.5	69	
15		43	50		23		25	32.5	33
	Third Series								
1		27	33		23			30	48
11		65	70		69.5		55	50	

connected with a source of fluid and a water manometer. The bladder was empty at the beginning of the experiment. The vesical volume was increased by adding 5 cc. increments of fluid until overflow occurred around the catheter. The intravesical pressure was read between each introduction of fluid when time had elapsed to permit the muscle to accommodate the added volume of fluid.

The readings of the bladder capacity were made once a week and the weights of the animals were recorded after each reading. It was found that the animals lost weight when the estrogenic hormone was administered.

Table 1 shows the results of 20 studies made on 11 different animals. The vesical volumes of the castrated cats were first read at weekly intervals for 2 or 3 weeks to determine the average vesical capacity. The estrogenic hormone was then administered. The hormone used was progynon-B which was kindly supplied by the Schering Corporation. The animals were given 2000 I.U. of this hormone in oil subcutaneously on the day on which the last control reading was made and again 4 days later. Each animal received 4000

I.U. in one week. One week after the administration of progynon-B was begun the vesical capacity was again determined. A significant diminution in the vesical volume occurred in 15 of the 20 studies. In several instances the bladder capacity was less than half the original figure. In 4 determinations there was no significant alteration in the vesical capacity and in only one of the 20 determinations was there an increase in volume. In several instances the injections of progynon-B were continued for 2 weeks and in one instance for 3 weeks. It was found that the animals did not do well under prolonged administration of the hormone, however, and in the series of determinations reported here the hormone was given for one week only. The vesical capacity was again determined at weekly intervals after the progynon-B was discontinued and it was found that the bladder capacity gradually returned to normal levels. In several of the animals, however, there was an increase following withdrawal of progynon-B, to values far above the normal capacity. Following this increase, the capacity gradually decreased to the normal values. The average vesical capacity, before the administration of the estrogenic hormone was begun, was 38.6 cc. This figure was obtained by averaging 45 determinations of vesical volume. The average capacity after one week administration of the hormone was 28.3 cc., or approximately 73.6% of the original capacity. In other words there was an average decrease of 26.4%. in vesical capacity. The average vesical capacity during the two to three weeks period following withdrawal of the estrogenic hormone was 43.3 cc. This figure represents a definite increase not only over the average volume during estrus, but over the original average control volume. The weights of the animals remained relatively constant throughout the experiment.

DISCUSSION

Our previous observations of the changes in vesical volume which occur in experimental animals during pregnancy and following ovulation together with the observation of Woolsey and Brooks that micturition volume diminishes during estrus led us to undertake this study. Twenty observations were made of the bladder capacity of 11 castrated cats following the administration of progynon-B. In 15 instances, or 75% there was a significant decrease in bladder capacity. In 4 instances or 20% there was no demonstrable change in capacity, and in only one instance, or 5% of the series was there an increase in vesical capacity. These results suggest that there is a relationship between the presence of the female sex hormone and the vesical muscle tone, and that in the presence of the estrogenic hormone the tone is increased.

The increase in vesical capacity which occurred in several instances following withdrawal of the estrogenic hormone suggests that the increase in capacity which follows delivery in the pregnant animal may be due to the same cause i.e., withdrawal of the estrogenic hormone.

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THE HISTOPATHOLOGY OF THE PITUITARY OF THE WHITE RAT INJECTED WITH FOLLICULAR HORMONE

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IT HAS NOW been definitely established that frequent injections of larger amounts of follicular hormones into mice and rats produce a marked hypertrophy of the anterior lobe of the pituitary with concomitant inhibition of growth and sexual function. The question arose next whether any histo-pathological changes occurred in the gland itself and whether it would be possible to correlate such changes with the previously observed changes in physiological function.

This problem has been investigated recently by several authors. Desclin (1) who injected 100 R.U. of dimenformon daily for 15 days into adult rats saw a diminution in the number of eosinophilic cells and a predominance of large cells with blue staining cytoplasm and containing large vacuoles filled with colloidal material. Hohlweg (2) who injected 100 R.U. of hydroxyestrinbenzoate daily into rats found changes in all three types of anterior lobe cells with swelling and diminished granulation in both types of chromophiles. Mazer, Israel and Alpers (3) reported an increase in eosinophiles in rabbits which had received large doses of follicular hormone. In rats which had received 100 R.U. for 100 days no histologic changes were found. In contrast to them Wolfe and Phelps (4), Wolfe and Chadwick (5) and Wolfe and Wright (6) found in both immature and adult male and female rats that following prolonged injections of estrin the chromophiles underwent degranulation and that all three different types of anterior lobe cells showed swelling, an increase in the size of the Golgi apparatus and vacuolization of the cytoplasm. In differential counts the number of eosinophilic cells was reduced to as much as one-third of that of the controls, the basophilic cells had nearly completely disappeared and the number of chromophobes was correspondingly increased.

Less complete histologic studies were reported by Cramer and Horning (7) who described in mice, the skin of which had been painted with a chloroform solution of estron, extensive hemorrhages and congestion of the anterior lobe. There was 'an excessive number of chromophobes.' McEuen, Selye and Collip (8) saw cavernous adenomas of the intermediate lobe and several times vacuolization of the posterior lobe. Burrows (9) found in one male mouse following cutaneous application of estrin for 483 days a pituitary hyperplasia with a normal posterior lobe, In the anterior lobe there was one type of cell only; "they had round or bluntly oval nuclei, surrounded by granular cytoplasm, which in some instances was faintly coloured by hematoxylin and in others by eosin." Hemorrhages were not found in the anterior lobe.

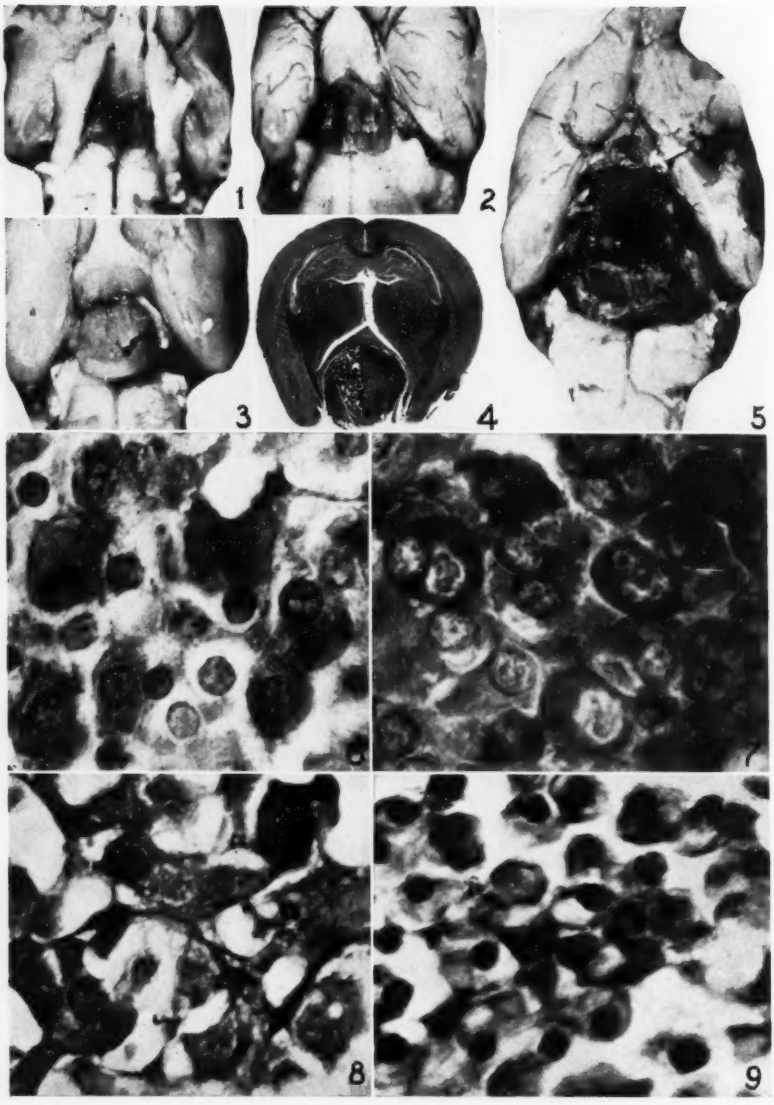
One of us (Z.) has previously reported a larger series of experiments of long-continued injections of folliculin into rats (10, 11). The following reports give a more detailed description of the histopathologic changes which occur under such experimental conditions in the pituitaries of these rats.

EXPERIMENTAL METHODS AND RESULTS

Methods of injection, staining and counting. The rats were injected twice weekly, young white rats of approximately 50 gm. initial weight being used. In the majority of experiments 5000 M.U. were injected subcutaneously as a single dose and the injection was repeated twice a week. In most of the experiments dimenformon (estradiolbenzoate) was used in an olive oil solution; exceptions are noted in table 1. The pituitaries were either fixed in Regaud's solution or in a solution of formaldehyde, U.S.P. (10% formalin). It was found that the latter method of fixation did not interfere with good differential staining, if the mounted sections, after removing the paraffin, were left over night in Zenker formalin solution. After treatment with iodine-alcohol and thiosulphate solution, as usually used for Zenker fixed material, the sections were stained with a modified technic used previously by Haterius. First the nuclei were stained with Harris' hematoxylin. Next the sections were left for about half a minute in the following solution: 2 parts of a 1% solution of ponceau de Xilidine in 1% acetic acid and 1 part of 1% acid fuchsin in 1% acetic acid. After being washed in distilled water the sections were fixed for about 5 minutes in a 1% solution of phosphomolybdic acid, washed in distilled water and stained for about one minute or less in a freshly prepared 1% solution of aniline blue. The sections were then blotted with bibulous paper, dried in the air and left for about 5 seconds, while being agitated, in absolute alcohol. They were quickly transferred through 3 changes of xylol and mounted in balsam.

The cells were counted under 2 mm. oil immersion lens with the help of a squared ocular micrometer. Each fifth section was counted of the total of serially cut and 5-micron thick sections. The counting followed along the longest diameter of the horizontally cut anterior lobe and all of the cells within the four central rows of squares of the Leitz 'Netzmikrometer' were counted. In the normal controls there was no difficulty in differentiating the three main types of anterior lobe cells; no attempt was made to count granulated and non-granulated basophiles separately. In rats which had been injected with large doses of dimenformon, however, the degranulation of basophiles and eosinophiles alike made differential counts more and more difficult with increasing amounts of hormone injected. With certainty only the exact amount of eosinophiles could be counted because they retained faint traces of granules. In order to differentiate between a faint blue staining chromophobe with enlarged Golgi apparatus and a large basophile in the process of degranulation the size of the nucleus and the presence of a well defined, red staining nucleolus were helpful as differential characteristics.

The intense vascularization following injections of more than 20 weeks' duration makes it impossible to deduce any conclusions from the weight of the gland as to the actually present secretory tissue. Furthermore, there is a gradual increase in the size of the interlobular cleft with accumulation of fluid, and, frequently, blood. It was for this reason that the attempt was made to express the relationship of anterior lobe, posterior lobe and cleft (referred to as 'cyst') in exact measures. The largest section of the horizontally and serially cut gland was selected, its outlines were drawn on thin tissue paper placed on the ground-glass of the photomicrographic apparatus at a magnification of 18 times and the sizes of the different parts were measured with the help of a planimeter. Though this method appears to be somewhat arbitrary, it never-



Figs. 1 to 5. PHOTOGRAPHS OF PITUITARIES OF A NORMAL CONTROL RAT AND OF 3 RATS WHICH HAD BEEN INJECTED TWICE WEEKLY WITH LARGE DOSES OF DIMENFORMON. Fig. 1. Female control rat. Fig. 2. Female rat injected for 16 weeks with 160,000 M.U. Fig. 3. Female rat injected for 38 weeks with 38,000 M.U. Fig. 4 and 5. Male rat injected for 42 weeks with 380,000 M.U. Fig. 4. Coronal section through brain; Van Gieson stained preparation. In the anterior lobe of the pituitary the enlarged interlobular cleft is visible; hypothalamus and third ventricle are compressed. Fig. 5. Enlarged pituitary with hemorrhages into anterior lobe.

Figs. 6 to 9. HISTOPATHOLOGICAL CHANGES OF ANTERIOR LOBE CELLS FOLLOWING DIMENFORMON INJECTIONS. Figs. 6 and 7 stained with a modified Haterius method, fig. 8 with Mallory's triple stain and fig. 9 with cresyl violet-eosin ×1100. Fig. 6. Male rat injected for 33 weeks with olive oil; 2 large basophilic cells together with polygonal eosinophilic cells and chromophobes. Fig. 7. Male rat injected for 25 weeks with 250,000 M.U. Most of the cells are swollen and rounded eosinophiles with markedly enlarged Golgi

TABLE 1. INJECTION OF DIMENFORMON INTO RATS 4 TO 5 WEEKS OLD, INJECTED TWICE WEEKLY

Injected weeks	Single dose M.U.	Total dose M.U.	Size in square mm. A.L.[1]	Cyst	P.L.[2]	Differential count % Acid. : Basoph.	Vascularity
			Male Rats				
Controls, 292 gm. mean weight			5.3	—	1.0	44 : 5	
11	Control, olive oil		4.4	—	0.8	38 : 11	
12	Control, olive oil		2.3	—	0.8	34 : 9	
33	Control, olive oil		4.5	—	1.7	32 : 12	
20	500	20,000	2.6	—	1.1	38 : 8	Percutaneous
32	500	32,000	7.1	—	—	31 : 11	Percutaneous
9	5000	90,000	4.1	—	0.7	32 : 10	+
11	5000	110,000	3.5	0.3	0.5	35 : 10	+++
11	5000	110,000	8.1	—	—	33 : 14	++
11	5000	110,000	4.3	—	0.6	30 : 13	+
22	5000	220,000	6.0	—	—	21 : 8	+
34	5000	340,000	13.2	2.6	—	25 : 13	+++(plus eschatin)
29	10000	580,000	8.1	2.3	0.5	24 : 10	+
30	10000	600,000	10.4	1.0	0.6	22 : 8	++
			Female Rats				
Controls, 180 gm. mean weight			4.4	—	0.9	35 : 7	
20	500	20,000	5.1	1.3	0.3	26 : 11	
38	500	38,000	4.1	—	0.6	28 : 12	
11	5000	110,000	3.9	—	—	37 : 13	+
17	5000	170,000	4.6	—	—	26 : 10	
22	5000	220,000	10.4	5.9	—	28 : 10	+
32	5000	320,000	13.2	4.6	—	27 : 10	+
41	5000	410,000	5.0	0.9	0.2	25 : 11	+++

[1] A.L.=anterior lobe.
[2] P.L.=posterior lobe.

Not included in this table are 11 male and 10 female rats in which differential counts were difficult on account of hemorrhages or post-mortem changes. The weight of their pituitaries has been included in table 2.

theless supplied figures for comparative studies, if the same method of fixation and staining was used. It should be noted that the size of the posterior lobe as given in table 1 does not indicate the largest horizontal diameter but only the diameter at the level of the largest cross section of the whole gland. In those rats which had received long-continued and large doses of hormone the enlarged anterior lobe had compressed and displaced the pars nervosa upward and backward and the latter did not appear in the largest horizontal sections which contained anterior lobe and cyst only.

The following table gives a summary of these measurements and of the differential counts. Only 17 out of a total of 38 experiments were used in this tabulation. The rest had to be discarded because some animals had died suddenly and rapid post-mortem changes interfered with proper staining. Some of those rats which had received excessive doses of hormone had large hemorrhages into the anterior lobe and the cleft and this 'pituitary apoplexy' might have been a cause of death. The relative degree of vascularity recorded in this table with the help of a plus system was determined by comparison with normal glands and other glands from injected animals.

apparatus. One basophilic cell is visible at the center of the first row. Fig. 8. Female rat injected for 24 weeks with 240,000 M.U. The pale staining of the section brings out a number of vacuoles within the cytoplasm of both types of chromophilic cells. Fig. 9. Male rat injected for 42 weeks with 380,000 M.U. Most of the small cells with pyknotic nuclei stain faintly red with eosin. The cytoplasm is condensed to a thin rim at the periphery and apparently compressed by the extremely enlarged Golgi apparatus.

Histopathologic changes. It has been pointed out before and is borne out again by the preceding figures that the number of repeated injections rather than the dose of follicular hormone injected determines the degree of enlargement of the anterior lobe. This statement, however, should be amplified by pointing out that apparently with 500 M.U. as a single dose the total amount injected has to be larger than 20,000 M.U. in order to produce structural changes.

These changes are hyperplasia of the chromophobes with hypertrophy of the anterior lobe, a gradual degranulation of the chromophiles, enlargement of the Golgi apparatus in all three types of cells with vacuolization of the cytoplasm, increased vascularity, enlargement of the interlobular cleft and finally hemorrhages into both anterior lobe and cleft. These histopathologic changes may be followed in their gradual development with increased doses of hormone injected if one subdivides the experiments into 3 groups.

Group 1. Injected from 9 to 12 weeks with a total maximum of 120,000 M.U. The anterior lobe is mildly enlarged up to about 1.5 times the normal size. There is a mild swelling of all 3 types of cells which are somewhat more rounded in their outlines. The number of dark staining, small nuclei is somewhat increased as compared with the normal controls. The number of eosinophiles is diminished to a level of 30 to 35% as compared with 43% in the male controls. The basophilic cells are well differentiated. The Golgi apparatus in both eosinophiles and basophiles appears normal. The pars intermedia and the pars nervosa do not show any pathologic changes.

Group 2. Injected from 17 to 30 weeks with a total maximum of 600,000 M.U. The anterior lobe is enlarged to about 2 times the normal size. All the 3 cell types show a markedly enlarged Golgi apparatus, which in the larger cells contains a coarse granular debris which stains faintly red. In a number of basophiles and eosinophiles small vacuoles appear in the cytoplasm which are distinct from the Golgi apparatus. The nuclei in these cells are well preserved, though somewhat larger and pale staining. The level of eosinophilic cells is diminished to 21 to 28%. The basophiles have undergone degranulation and may be differentiated from pale blue staining chromophobes only by the size of their nuclei, the large red staining nucleolus and the size of the markedly enlarged Golgi apparatus. There is a marked increase in vascularity without, however, intralobular hemorrhages. The pars nervosa is somewhat small, compressed by the enlarged anterior lobe. Histologic examination does not show any marked changes and there is no increase in vascularity.

Group 3. Injected for more than 32 weeks with a total maximum of 780,000 M.U. In this group 7 rats have been included which are not recorded in table 1 because severe hemorrhages had disturbed the cytologic picture to such an extent that differential counting was impossible. The anterior lobe is enlarged to three to four times the normal size. The interlobular cleft is markedly widened and contains large, rectangular crystals (hemoglobin?) besides red and white blood cells and large scavenger cells filled with the debris of blood cells. There is a marked increase in vascularity which has led to final hemorrhages into the anterior lobe itself. The increase in connective tissue fibers and the presence of scavenger cells filled with dark staining pigments indicate repair processes which followed previous hemorrhages. All the 3 different types of cells have markedly deviated from the normal picture. Their Golgi apparatus is maximally dilated and leaves only a small rim of the compressed cytoplasm. There is a resemblance to the 'signet ring' cells of the castrate hypophysis. The eosinophilic cells are maximally degranulated and are sometimes only recognized with difficulty.

Their level is below 25% of the total count. Most of the basophiles are degranulated but may still be recognized as such by the size of the Golgi apparatus and their nuclei. The pars intermedia and nervosa are very small and compressed by the enlarged anterior lobe. There is an increase in glia fibers in the posterior lobe and the number of nuclei is diminished as compared with controls. The enlarged anterior lobe has also compressed the adjacent nervous structures. The chiasm and the floor of the third ventricle are mainly affected. The latter in some cases is pushed upwards and the third ventricle itself is compressed to a narrow fissure. There is surprisingly no marked gliosis in the compressed tissue, but the hypothalamic nuclei show signs of progressed degeneration in the form of vacuolization of the cytoplasm and shrinkage of nuclei of its neurons. In some cases both supra-optic nuclei are involved predominantly. This may be due to retrograde degeneration following atrophy of the pars nervosa and compression of the infundibulum. But interference with blood supply should also play an important rôle. In 2 rats thrombus formation which had been partly organized was found in the anterior cerebral arteries which had been compressed by the enlarged pituitary. In the frontal lobes, supplied by these arteries, several old areas of softening were found, partly infiltrated with calcium salts and organized.

In one rat which had been injected for 53 weeks with a combination of dimenformon and benzpyrene an area of softening was found at the base of the left frontal lobe. Though this male rat had received 53,000 M.U. only during 53 weeks, the anterior lobe measured 20.5 sq. mm. in its largest diameter. In another male rat which received the same combination of a carcinogenic substance with dimenformon and the same amount of units, the hypophysis weighed 300 mg. It did not seem, however, that the addition of benzpyrene intensified the effect of dimenformon, since in experiments of shorter duration, 20 weeks, the hypophysis of these rats was not larger than the hypophysis of rats injected with dimenformon only.

It should be pointed out here that in the 3 male rats which were injected with olive oil only as controls, there was an increase in the number of basophiles to a level from 9 to 12% as compared with 6 to 7% in the non-injected controls with a corresponding decrease in the number of eosinophiles. Recently Reiss, Epstein and Gothe (12) reported that injection of 1 cc. of olive oil daily into rats for a period of from 14 to 18 days was followed by an increase in the number and enlargement of basophilic cells. This indicates that the increased number of basophiles in the anterior lobes of estrin-injected rats should be attributed rather to the effect of the olive oil in which the hormone was dissolved than to the effect of dimenformon.

The hyperplasia of the chromophobes in most of the anterior lobes was rather uniform. In 6 rats, however, which had been injected with total doses of 120,000 to 320,000 M.U. there was a central accumulation of chromophobes in tumor-like form, compressing the outer layers of the lobe which consisted of mixed cell types. Such an arrangement has been described before and has been referred to as (tumor) or (adenoma.) It should be emphasized, however, that frequently one finds in normal rat pituitaries such a central accumulation of either eosinophiles or chromophobes and a denser structure of the outer zones which contain comparatively more basophiles than the center. Such an arrangement indicates that there is a more active stimulation of cellular growth in the center of the lobe and the same applies to the con-

densed islands of chromophobes in the pituitaries of the injected rats. One, therefore, should avoid speaking of 'tumors' as long as there is no neoplastic transformation of anterior lobe cells and one should rather describe the phenomenon observed in these experiments as "hypertrophy of the anterior lobe with hyperplasia of the chromophobic cells."

DISCUSSION

If one compares the results of these experiments with those reported by Wolfe and his coworkers (4, 5, 6) the parallelism is obvious. In both groups of experiments there was an increase in the size of the anterior lobe roughly proportional to the amount of follicular hormone injected. This hypertrophy was due to a hyperplasia of chromophobes with concomitant reduction in the number of eosinophiles. This agreement, however, is only a qualitative one. Though a comparison is difficult because Wolfe and his coworkers gave daily injections and smaller amounts of estrin and because they report R.U., it nevertheless appears that the reduction in the number of eosinophiles and the disappearance of basophiles was much more marked than in our own experiments. These quantitative differences, however, may be explained partly by differences in staining technique. Wolfe uses a method described by Cleveland and Wolfe (13) in which the eosinophiles were stained with erythrosin after long mordanting with potassium dichromate. In normal glands there are good agreements in the number of eosinophiles as compared with the method used in our experiments. But, it seems, judging from the experience of one of us (W.) that with beginning degranulation better results in staining eosinophiles can be obtained following Zenker fixation and staining with the modified Haterius method. The discrepancy in the number of eosinophiles, however, may be due also to the fact that Wolfe and coworkers injected daily 200 R.U. of dihydro-estrinbenzoate and a much larger total number of times than in our own experiments, thus aggravating the effect of the follicular hormone. The discrepancy in the number of basophiles may be explained on technical grounds and by the fact that we tried to identify the basophiles which were already markedly degranulated, while the other authors counted basophiles with definite, blue staining granules only.

The failure of Mazer, Israel and Alpers (3) to demonstrate the decrease in eosinophilic cells and the histopathologic changes in all the 3 types of cells of the anterior lobe stands alone among all the other observations which have been reported and may be accounted for by the relatively small dose injected in their experiments.

Our experiments have emphasized again the fact that following injections of large doses of estrin the changes in growth and sexual function are not necessarily accompanied by an increase in size of the anterior lobe. However, they are invariably paralleled by a diminution in the number of eosinophilic cells. On the other hand the increase in size of the anterior lobe in the experiments of groups 2 and 3 is not only produced by a hyperplasia of the chromophobes, but at the same time the increased vascularity and the extension of the interlobular cleft contribute considerably to this tumor-like enlargement.

The compression of the pars nervosa, of the pars intermedia and of the hypothalamus and the interference with the vascular supply of the brain observed in group 3 should caution one not to ascribe all the changes in physiological function to a disturbance of the hormonal equilibrium only. Our experiments demonstrate that the maximal amount of dimenformon which may be injected without producing a hypertrophy of the anterior lobe is 110,000 M.U. in male and 200,000 M.U. in female rats.

The following table 2 will give a comparison between our own experiments and others reported in the literature. Due to the fact that R.U. are used by some and M.U. by others and that the amount of total units injected varies just as much as the number of total injections, it is difficult to compare the observations of different authors.

In the following table our own results are compared with those of Wolfe and Wright (6) who injected 200 R.U. of dihydro-estrinbenzoate daily. Our normal values refer to adult male and virgin female rats of average weight of 292 gm. and 180 gm., respectively.

TABLE 2. COMPARISON OF DIFFERENT OBSERVATIONS ON THE PERCENTAGE AMOUNT OF EOSINOPHILIC: BASOPHILIC CELLS IN THE ANTERIOR LOBE OF THE RAT PITUITARY FOLLOWING ESTRIN INJECTIONS. (MEAN VALUES.)

Male Rats

Normal Rats	Rasmussen (14) 36.8:10.9	Sutton & Brief (15) 43.5:9.8	Weil & Zondek 43.8:5.3	Weil & Zondek olive oil inject. 34.7:10.7	
Injected times	18–24	34–60	64–84	132	306
Wolfe and Wright	—	—	—	12.2:0	9.7:0
Weil and Zondek	32.5:11.8	22.3:12.0	23.5:13.0	—	—
Weight mg.	11.5	51	77	45	41

Female Rats

Injected times	Normal (16)	30	22–28	30–52	80	64–84
Wolfe and Wright	34.2:4.1	15.8:.1	—	—	14.1:.1	—
Weil and Zondek	35.2:6.6	—	32.0:13.0	26.7:10.3	—	26.0:11.3
Weight mg.	12.0	30.2	10.5	46	33.3	70

SUMMARY

Injections of dimenformon into white rats continued twice weekly over long periods produced the following changes in the pituitary.

Total maximal dose 120,000 M.U. injected during 9 to 12 weeks. The anterior lobe was enlarged to about 1.5 times its normal size and there was a mild swelling of the cells of the 3 different types. The proportion of eosinophilic cells was 35 to 30% as compared with 38 to 32% in controls injected with olive oil only.

Total maximal dose 600,000 M.U. injected during 17 to 30 weeks. The anterior lobe was enlarged to about twice its normal size. There was a mild increase in vascularity. In all the 3 types of cells which were mildly swollen

the Golgi apparatus was markedly enlarged and contained a coarse granular debris. The proportion of eosinophilic cells was diminished to 21 to 28%.

Total maximal dose 780,000 M.U. *injected during 32 to 63 weeks.* The anterior lobe measured 3 to 4 times its normal size and obtained a maximal weight of 100 mg. The interlobular cleft was markedly widened. There was a very marked increase in vascularity which in a number of cases led to intralobular hemorrhages and death. The cells of all the 3 types were swollen, their Golgi apparatus was maximally dilated and most of the chromophiles were degranulated.

The pars intermedia and the pars nervosa were not directly affected by the hormone injections. They were compressed by the enlarged anterior lobe in 2nd and 3rd groups. The pars nervosa in the advanced cases showed a loss of cells and an increase in glia fibers.

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SPONTANEOUS PERSISTENT ESTRUS IN A STRAIN OF ALBINO RATS

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In 1921 Evans and Long (1) reported that 7 rats in a group of about 800 exhibited unusually prolonged vaginal cornification, ranging from 2 to 21 days. In each case, this was correlated with the persistence of an entire set of large ovarian follicles, none having ruptured and luteinized. Persistent estrus, which appears to be identical to this, has occurred spontaneously at an early age in such a large number of rats in my colony that an intensive study of the condition has been undertaken.

The strain of rats in question is descended from two pairs of albinos from Dr. J. S. Nicholas' colony in the Osborn Zoological Laboratory in Yale University. The stock was originally Wistar albino, but it had been outcrossed on 4 occasions with Wild Gray and once with the Smith Engle Berkeley hooded strain before it was brought to this laboratory in 1932. Close inbreeding was not practiced until 1936, but since that time brother-sister matings have been the rule, with attempted selection of a line of good breeders.

The general health of the colony is good. Pulmonary infections which are so frequently encountered in rat colonies have been reduced to a low level during the past 2 years as the result of improved living conditions. The cages are constructed of wire mesh throughout, in order to give optimum ventilation and cleanliness. As a rule, not more than 3 adults are kept in one cage, especially during the summer months when the room temperature unavoidably reaches 90° F. or more. The ration is Purina dog chow, supplemented by cabbage. Lactating females and newly weaned young are given whole milk in addition to this diet.

Irregularities in breeding performance of the females were first observed during the spring of 1936. Parturition was often delayed one or more days and was frequently very difficult. In many of these instances, full litters of fetuses were delivered dead in their unruptured membranes. The yolk sac epithelium was characteristically necrotic and was usually firmly adherent to an epiplacental white plaque, a fibrinous mass of leucocytes. These pathological changes were found to appear shortly after the outer wall of the yolk sac had ruptured and its cavity had become confluent with the uterine cavity. On other occasions, delayed parturition resulted in the delivery of only one or two living, but decidedly oversized fetuses. Abnormal litters of both varieties were sometimes produced by a single female in a series of pregnancies. Early sterility was characteristic, not only of animals which had delivered abnormal litters, but of others which had mothered one or more litters of normal young.

A study of the vaginal smears of these sterile animals showed a condition of more or less continuous cornification.[1] In many cases cornification was constant over periods of a month or more, interrupted only by occasional diestrous intervals of 1 to 4 days. The ovaries of these animals contained, in each instance, a full set of large follicles, the average diameter of which was about 0.8 mm. As in the cases described by Evans and Long (1) the granulosa was usually healthy in appearance, but was often folded upon itself here and there, with tufts of capillaries projecting from the tunica interna into the clefts thus formed. New corpora lutea were conspicuously absent in most instances, yet in 2 individuals luteinization has been observed to occur at the end of a prolonged estrous period.

The tubes were often abscessed or closed by scar tissue, although in a number of animals only one tube was affected. The uteri showed evidence of varying degrees of inflammation; from a chronic, low-grade endometritis to an extensive pyometra involving one or both horns. In several of the older rats in which estrus had continued for many weeks, the histological picture was similar to that which various authors (2, 3, 4, 5) have observed, following the long-continued injection of estrogens into rats and mice. Glandular abscesses and epithelial pearls were abundant and occasionally metaplasia was to be seen in the lining epithelium itself. In one rat (370.1), killed when she was about a year old, the rostral end of one horn of the uterus was cornified. Unfortunately, the cervices of such individuals were not preserved. The vaginal epithelia were typical of full estrus.

As all of these initial observations had been made on older animals, there was no way of estimating the age at which persistent estrus had first appeared. A group of young rats has since been studied by daily smears, beginning at about the 75th day. Complete records have been made on 33 of these animals through the 200th day. Of these, 25 have shown some degree of persistent estrus before that age, while 6 have maintained unquestionable, regular cycles. The other 2 were fairly regular, but occasionally remained in full vaginal cornification for 48 hours. The persistent estrous condition has become constant or has recurred consistently in 20 of the first group, the remaining 5 apparently having recovered. Of the 20 recurrent cases, 2 animals were clearly aberrant before 100 days, 12 more before 150 days and another 6 before the 200th day was reached.

The extent of continuous cornification has varied between doubtfully abnormal 48-hour periods and an extreme of over 90 days. The vaginal sequences of 9 selected individuals have been plotted in figure 1. It will be seen that the cycles of 782.3 exhibited only slight irregularities. Those of 804.3 were regular and of normal duration until her 148th day when they became quite erratic. The latter half of the record of 614.3 is of especial interest, for it illustrates a pseudocyclic sequence which characterizes certain animals. Although such cases exhibit diestrus at frequent intervals, luteinization does not ordinarily occur. In this rat, for instance, no corpora appeared in the ovaries, even though a short 'cycle' of normal length had occurred only a few

[1] Smears were made by lavage with physiological saline introduced by a pipette.

days before she was killed. Sporadic occurrence of cycles of normal duration, interposed between periods of prolonged cornification is illustrated by 832.2 and 513.1. In many instances, the interspersed cycles were not found and the prolonged cornification was interrupted by only short periods of diestrus. This may be seen in the later history of the animals just mentioned and also in the sequences of 537.5 and 628.1. The remaining two examples (513.2 and 571.2) are presented to illustrate an effect which was observed when copulation occurred during a persistent estrous phase.[2] Diestrus appeared within 48 hours, but was of short duration and a brief period of estrus transpired. An interval of prolonged diestrus comparable to pseudopregnancy followed and, in turn, a short cycle preceded the resumption of prolonged cornification.

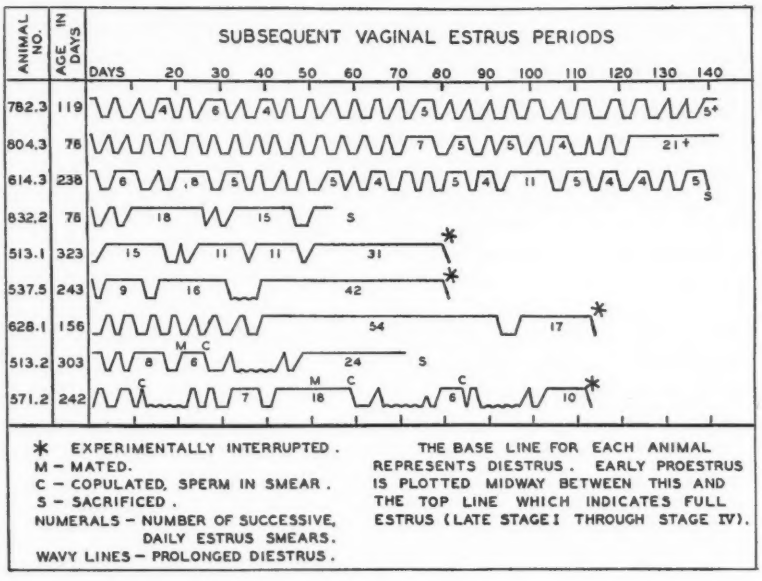
ANIMAL NO.
AGE IN DAYS
SUBSEQUENT VAGINAL ESTRUS PERIODS
DAYS 20 30 40 50 60 70 80 90 100 110 120 130 140
782.3 119
804.3 78
614.3 238
832.2 78
513.1 323
537.5 243
628.1 156
513.2 303
571.2 242
* EXPERIMENTALLY INTERRUPTED.
M – MATED.
C – COPULATED, SPERM IN SMEAR.
S – SACRIFICED.
NUMERALS – NUMBER OF SUCCESSIVE, DAILY ESTRUS SMEARS.
WAVY LINES – PROLONGED DIESTRUS.
THE BASE LINE FOR EACH ANIMAL REPRESENTS DIESTRUS. EARLY PROESTRUS IS PLOTTED MIDWAY BETWEEN THIS AND THE TOP LINE WHICH INDICATES FULL ESTRUS (LATE STAGE I THROUGH STAGE IV).

FIG. 1.

In view of the fact that rather severe infections of the uteri and tubes had been noted, bacteriological studies of a few animals were made. Uterine scrapings were obtained, under aseptic conditions, from individuals which had been in heat for varying lengths of time, and also from animals which were in diestrus. The scrapings were streaked on blood agar plates. In 11 cases out of 12, in which the vaginas had been cornified from 3 to 20 days and were still cornified at the time the scrapings were made, heavy bacterial growth appeared on the plates within 18 hours. In one instance, *B. pyocyaneus* was obtained in nearly pure culture, but in the others mixed cultures of gram negative organisms resulted. The 12th case was negative. Five other animals, in diestrus after prolonged heat periods, yielded negative results.

[2] The male was always removed from the cage as soon as sperm were found in the smear.

The same was true for 2 normal animals in diestrus. The uteri of 4 rats in stages I and II were likewise bacteriologically sterile.

The continuous treatment of rats and mice with large amounts of estrogenic substances has been shown to encourage bacterial growth in the uterus in like fashion (6). Gardner and Allen (7) observed that in mice receiving large weekly doses of an estrogen for several months, inflammatory reactions were not to be found in segments of the uterus which had been transplanted to a subcutaneous position before puberty. It thus seems not improbable that, in the persistent estrous rat, the continuous supply of estrin enhances bacterial growth and that this in turn is responsible for the inflammatory condition in the tubes and endometrium. The epithelial metaplasia may or may not be directly associated with the infection.

No information has yet been obtained regarding the relationship of the abnormal vaginal sequences to the production of abnormal litters. Certainly it would be reasonable to suppose that any inflammatory reaction set up in the endometrium would tend to produce sterility, even if there were occasional ovulation and fertilization of ova. This could easily account for the implantation of only one or two embryos. The occurrence of a plaque of leucocytes on the placenta, in the still-born full litters, would suggest that bacterial infection is operative here as well. In this regard, it is significant that the yolk sac abnormalities and the plaque have not been recognized until after the outer wall of the yolk sac has ruptured and it communicates with the uterine cavity. Nevertheless, one cannot lose sight of the fact that delayed parturition itself is a well recognized accompaniment of various experimental procedures which disturb the hormone balance during pregnancy. It may be unwise to assume that before the beginning of an abnormal pregnancy the mother had exhibited any endocrine disorder, whatsoever.

Endocrine dyssynergia, probably very similar to that which had been outlined here, has been observed in other colonies. Probably Evans and Long (1) would have observed many more cases of prolonged cornification had they followed their rats throughout life. Asdell and Crowell (8) observed that out of a group of 21 rats, 8 animals exhibited continuous cornification when examined at about 11 months of age. Six months later, that number had increased to 10, only 5 rats were cyclic and the other 4 surviving animals were anestrous. In the Albany (A-S) strain in which a condition of 'vaginal hyperestrinism' (9) has recently been described, "an occasional animal was observed in which fully cornified smears persisted for long periods" (10). In many other animals "the actual length of time during which the smear contained only cornified epithelium was not materially increased, but the rats failed to pass into a normal diestrus" (9). Most of these studies were made upon rats which were over a year old. No information is given regarding the frequency of prolonged cornification in younger animals, other than the statement (10) that it 'may occur' in them.

It becomes evident that these phenomena are in great measure a function of age. Since certain rats become anestrous without undergoing a term of prolonged estrus, as Wolfe, Bryan and Wright (10) observed, then one must

inquire whether there are not hereditary and (or) environmental factors which cause other rats (for example, most of those in the local strain) to pass through long intervals of continuous vaginal cornification with persistent follicles. Or is the difference merely one of statistical variation?

SUMMARY

A strain of rats is shown to have developed a strong tendency toward persistent ovarian follicles and accompanying vaginal cornification. This condition appears early in life in a high proportion of the population, becoming increasingly prevalent as the animals become older. In the same population, there is also a pronounced tendency toward delayed and difficult parturition. The continuous supply of estrin in animals which remain in estrus for long periods is at a sufficiently high level to produce changes in the uterus and tubes, simulating those which result from the continuous administration of large amounts of estrogenic substances.

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DETERMINATION OF THE pH OF THE VAGINAL FLUID AS AN OBJECTIVE METHOD OF OBSERVATION IN ADMINISTRATION OF ESTROGENS

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A CHANGE IN pH of the vaginal fluid in castrate human beings follows the administration of estrogens.[1] There is an alteration from alkaline to the acid phase, until the normal pH is attained. Employing a universal indicator, and by adding several drops to the fluid sample, a color reaction can be perceived. With adequate dosage of estrogens administered, the normal pH value will be attained and subjective and objective improvement will be noted. Not infrequently, this is predictable, when the change to the acid phase in the vaginal fluid results.

Before administration of estrogens, the mucosa of the vestibule, vagina and portio cervicalis appears injected, superficial pin-point ulcerations of the mucosa can be seen; muco-seropurulent discharge is present. The cervix itself is not infected, nor the seat of an erosion or endocervicitis, infection and parasitic infestation having been excluded clinically and upon examination of smears and hanging-drop preparations. Since no topical treatments had been given, and medicated douches were omitted, it would seem that the subjective and objective improvement which follows the administration of estrogens is associated with the change of the pH to normal or acid phase.

Similar changes in pH are demonstrable in group-cases of hypo-ovarianism, more especially, the menopause group. A change in pH to the acid phase seems to follow subjective as well as local genital improvement, after administration of adequate amounts of estrogens.

These observations have been followed for a period of 3 years, and the pH determination has remained as a routine method in clinical investigation, and as a guide in treatment with estrogens.

Papanicoloau and Shorr have demonstrated the clinical value of vaginal spread examination in human beings. Vaginal spreads made simultaneously with hydrogen ion determinations on the vaginal fluid, after administration of estrogens, show a change in the cytology. The acid phase of the pH seems related with cytology of the high follicular phase. The change in pH is closely associated with improvement in cytology and amelioration of symptoms. These invariably follow concomitant changes in pH and vaginal smear.

Further study is presently being conducted to elucidate whether the

[1] Progynon-B was generously supplied for this study through the kindness of Dr. W. Stoner of Schering Corporation, Bloomfield, New Jersey.

changing cytology or the fluid part of the vaginal contents is related to the change of the pH in the vaginal fluid.

Experimental studies in the rat, constituting a subject of another report, seems to indicate that pH of the vaginal fluid is related to the cytology of its contents; the castrate remains in alkaline phase and alteration to the acid phase of pH follows upon administration of estrogens and with return of the cycle. This experimental observation needs further study to determine whether the fluid element of the vaginal content plays a part.

Reding (1) observed the effect on the pH of the blood in human beings after administration of estrone. There were no changes following injection of large amounts of estrogens in normals, and a profound change to the alkaline phase was induced in castrate, menopause and hypo-ovarian women. Objective improvement of non-infective erosion and endocervicitis after hormone therapy has been recently reported by Wollner (2).

SUMMARY

A method of observation and a guide in treatment with estrogens in human castrates, menopause and hypo-ovarian groups, is offered. It is a simple test, easily carried out and suitable as an office or clinic procedure.

I am deeply indebted to Dr. Raphael Kurzrok of the Presbyterian Medical Center, New York, N. Y., for his encouragement and stimulating advice, and to Drs. Koplowitz and Oginz for their broad vision in creating a departmental laboratory and gynecological endocrine clinic.

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CASE REPORTS

PITUITARY CACHEXIA

REPORT OF A CASE

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IN THIS communication we report a case of pituitary cachexia diagnosed during life and confirmed at autopsy. The pathologic proof of this syndrome is rare, especially in the English language publications (1). According to Davis and Postle (2) there were 58 proven cases up to the end of 1937. We have been able to find 5 more reported during the year 1938, bringing the total in the available literature up to 63. Incidentally this case was the only one on record at the New York City Hospital in 28 years with 189,314 admissions and 5,392 autopsies.

We feel justified in reporting this case because *a*), it is a rare condition; *b*), it gave evidence of a long duration with involvement during early life; *c*), it exhibited a massive cyst extending into the cerebrum and completely obliterating the anterior lobe of the pituitary and *d*), the diagnosis was verified during life.

REPORT OF CASE

A.S. a Puerto Rican laundryman 37 years old was admitted to the New York City Hospital September 25, 1930 as a transfer from Bellevue and died on November 13, 1930. His chief complaint was severe frontal headaches that had incapacitated him for the last two months.

In Bellevue he was not very cooperative and seemed to have a poor memory but in the City Hospital his mentality was recorded as fair. Outside of a questionable case of typhoid at 10 years of age he had never been sick. He denied any form of venereal disease.

On physical examination he was noted to be infantile in appearance, about 4 ft., 8 in. in height, with a feminine build. His hands were long and tapering and his frame was small and light. There was no pubic or axillary hair nor any visable elsewhere on his body except on the head where it was thin and silky. The mons veneris was markedly feminine and the genitals were small and infantile.

He had no complaints about his eyesight and the results of an examination of the eyes were negative. For several months previous to his admission he had noticed polyuria with three or four voidings each night.

In the hospital his temperature was subnormal, ranging from 97 to 98.4°. His blood pressure was also low, the lowest noted being 60/40 and the highest 90/56. The first B.M.R. was −39 and the last one was −27. The x-ray of the sella turcica showed some enlargement without any destruction of the clinoid processes. Wassermann test result was negative and the fasting blood sugar was 90.

He was put on whole pituitary extract, two grains T.I.D. by mouth for several days. He said the headaches were improved. From Oct. 10, 1930 to Oct. 21, 1930 he was given antuitrin intramuscularly every other day and during that time his headaches were relieved. However the supply of antuitrin in the hospital ran out and he was returned to whole pituitary gland by mouth. This was given at the rate of 5 grains T.I.D. The severe headaches returned and the patient asked to be put back on the injections. On October 20 thyroid extract ¼ grain was given T.I.D. On October 31 his headaches were getting worse. At this time he passed large quantities of urine with a low specific gravity. Later he became incontinent and November 13 he died in his sleep.

PATHOLOGIC REPORT

Autopsy 3587. The body was that of a Puerto Rican of rather uncertain age. The stature was small. The limbs were thin, the bones were small and the fingers were long, narrow and pointed. The head hair was thick curly and black. There was no hair on the face, in the axilla, on the body or on the pubis. The mons veneris was prominent. The external genitalia were infantile in type and each testis could be palpated in the scrotum. The skin was smooth, soft and pale. The genital organs were normal except that they were small in size.

Fig. 1. (Left) INFERIOR SURFACE OF THE BRAIN. The cyst appears on the under surface of the right frontal lobe.

Fig. 2. (Right) HORIZONTAL SECTION OF THE BRAIN SHOWING THE LARGE CYST EXCAVATING THE RIGHT FRONTAL LOBE.

Brain. The skull cap was very thin. The dura was distended and tense. The brain itself appeared swollen and the convolutions were flattened. On lifting the anterior portion there was found a large cyst occupying the pituitary fossa and eroded into the body of the sphenoid. The clinoid processes were absent. On opening the roof of the sphenoid the cyst wall was broken and discharged a thin brownish fluid. The stalk appeared normal. There was a stalk running from the cyst occupying the pituitary region to the inferior surface of the right frontal lobe and joining a large cyst which appeared on the inferior surface lateral to the straight sulci. Both frontal lobes

appeared cystic, the right more than the left. On injection of the vessels of the base of the brain the cyst on the left distended slightly. On horizontal section of the brain there was a large cyst occupying the entire white portion of the right frontal lobe which had a very thin membrane and was tightly attached to the brain tissue. This large cyst communicated with the mass in the pituitary region and by the thin stalk described above. The cyst had distorted the corresponding anterior horn of the ventricle. Both ventricles were moderately dilated. The foramen of Monro was markedly dilated.

HISTOLOGY

Pancreas. The pancreas was normal in appearance.

Thyroid. The acini appeared about the same size and were all filled with colloid. The epithelium was low and flattened. The interstitial connective tissue was very scanty. Inflammatory cells were absent. There was no evidence of activity of the acini cells.

Testes. Connective tissue of the testes appeared relatively increased in amount. The seminiferous tubules were small. The majority of the cells were cells of Sertoli. Many of the tubules contained onlythis type of cell. In others there were a few cells of seminiferous type, some of which had reached the stage of primary spermatocytes, but no cells of more adult character were present. Mitoses were completely absent.

Anatomical diagnosis. Pituitary cyst with complete inactivity of the thyroid and underdevelopment of the testes with only the very early phase of spermatogenesis.

DISCUSSION

This case is an example of the Lorain type of infantilism showing immaturity in both the skeletal and the sexual systems. One can justifiably assume from this that the growth stimulating and the gonadotropic factors of the anterior pituitary gland were deficient before the adolescent period and as the pathologic findings point to a long duration of the cyst of the pituitary one is within his rights in believing that the cyst was present sometime before puberty and is the etiologic factor in the case.

Cysts of the pituitary are one of the recognized causes of pituitary cachexia although they are not reported as frequently as other causes, especially the classical form of Simmonds which is an atrophy of the anterior pituitary following parturition.

The cyst probably originated in the pars intermedia as do most of those reported. It can be considered both an intra- and a suprasellar tumor. After an examination of the extent of this growth one is surprised at the lack of accompanying symptoms throughout the many years of its existence. The man's history stated that he lived a relatively comfortable life until his last few months.

The anterior lobe was completely destroyed. Here also it is surprising that any physiologic relationship between this and the posterior lobe or the general system could be possible. All one could say was that a small quota of the anterior lobe secretion must have reached its normal destination up to a late date in his life.

The x-ray of the sella, while positive for a growth about the structure, gave a very incorrect idea of the size of the cyst and of its destructive action on the clinoid processes and the walls. Again it was surprising after making

the pathologic examination that the x-ray could fall so short of giving the true picture, especially in relation to the bony structures.

The value of treatment in this case may be given consideration. A priori it is a surgical condition of the pituitary. The release of the tension in the cyst by puncture with the possibility of the removal of the sac would have been essential if the diagnosis had been more accurate. As someone has said after an examination of the pathologic changes in the anterior lobes of fatal cases it is hard to believe that a cure could be obtained by any method of therapy now at hand. However, substitution therapy has its place even in such a case as this. The growth hormone could have been given throughout adolescence. The gonadotropic factor was definitely needed as proven by the histology of the testes and might have been required continuously. The condition of the thyroid gland is good evidence of the absence of a normal stimulus and coincides with the low basal rates. Whether the growth and gonadotropic factors together would have been capable of keeping the thyroid normal in this case is hard to say with our present knowledge. The thyrogenic fraction of the pituitary might be specific in this type of thyroid inactivity as a number of recent reports in the literature mention that this type of patient does badly on thyroid extract.

From the information we received through the autopsy it does not seem that the results obtained from medication in the hospital are to be given much weight. It is tempting to believe, however, that the use of pituitary extract parenterally relieved his headaches when it did not do so by mouth. Likewise the improvement in the metabolic rate might be attributed to the therapy yet the post-mortem findings weaken these assumptions.

SUMMARY

A case of pituitary cachexia produced by a massive cyst of the pituitary gland proven by autopsy is reported. The diagnosis and treatment of the case and its relationship with some of the other endocrine glands is discussed.

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ACROMEGALY

CASE REPORT WITH AUTOPSY FINDINGS

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ACROMEGALY is not of common occurrence according to Putnam and Davidoff (1). These authors state that "The diagnosis has been made only 14 times among the 210,094 cases admitted to the Boston City Hospital in six years from 1930 to 1935 inclusive. Males and females are about equally affected. It may occur in the black and yellow races as well as the white."

I had the opportunity of observing the patient here reported develop the disease and to follow its course until death.

CASE REPORT

History. S. Z., a Russian Jew, aged 28, married, entered Harper Hospital July 21, 1919, complaining of pain and swelling of the right testicle. This began 5 days previously while working at his occupation of body-maker in an automobile factory, and came on immediately after using the apparatus for lifting the body onto the assembly line. He had been treated 21 months previously for a gonorrheal urethritis of 4 months' duration.

Other than the fact that one brother had distinct acromegaloid features (seen by myself), the family history was negative. The patient was the father of two children.

When he was examined at this time the features were normal, the hair was dark brown, his weight was 165 lb. (74.8 kg.), height 68 in. (172.72 cm.), (fig. 3). The voice was normal. The orchitis and epididymitis of the right testicle were the only abnormality found. On August 5, 1919, a right-sided epididomyotomy was done, freeing about 2 cc. of straw-colored fluid and a small amount of pus. The fluid was not cultured on gonococcus media and no growth was obtained. He was discharged from the hospital 13 days later, the testicle still being edematous but not tender. He was seen again on May 7, 1921 for a fracture of the right clavicle as a result of an automobile accident. His general appearance was the same as when seen 2 years previously. Healing of the fracture was without incident.

He came to the office July 19, 1926, stating that 8 months previously (December, 1925) he noticed that his face and hands were becoming larger and his voice was deeper. He had no headache, change in vision or in the size of his feet. His complaint was difficulty in eating because the jaws did not meet as they formerly did. The lower set of teeth were painful and felt as if there was insufficient room for them. These symptoms were of 3 months' duration. He also stated that while he always consumed large quantities of water, much more than the rest of the family, his thirst had increased with the onset of the present trouble. He consumed from 8 to 10 glasses of water at each meal and a like number between meals. While on a picnic a few weeks previously he consumed 32 glasses of tea and 10 bottles of beer. He had

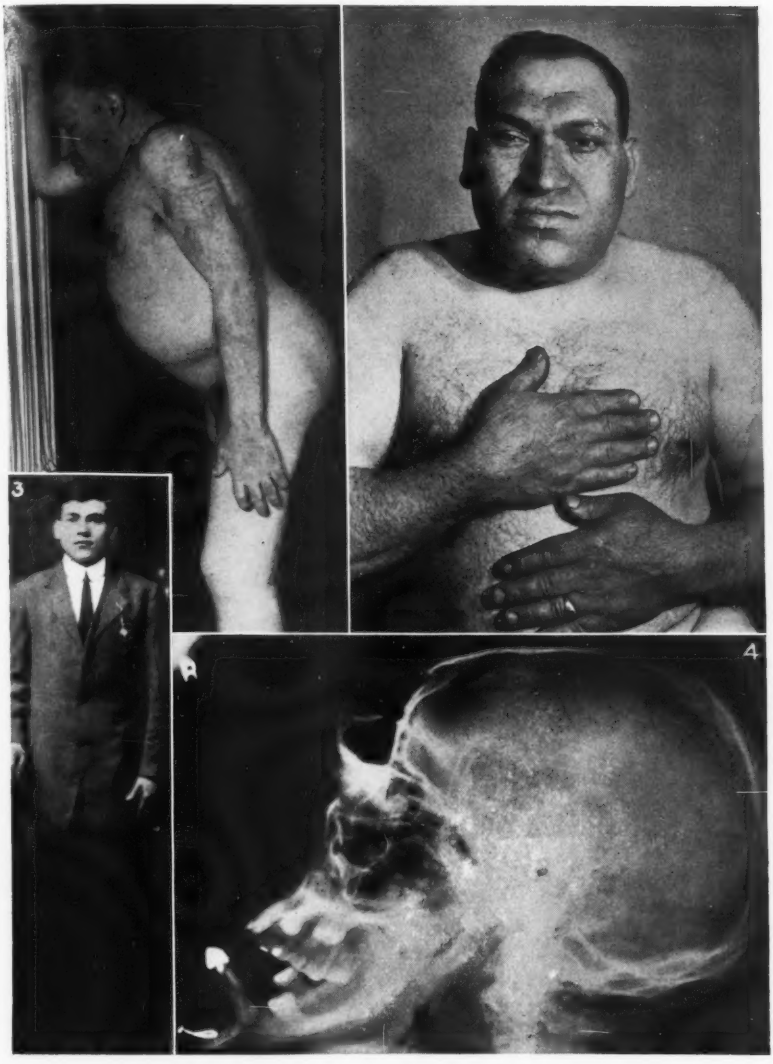

Fig. 1. Patient 2 days before death. Fig. 2. Patient 2 years after onset of acromegaly at age of 35. Fig. 3. Patient at age of 22, before onset of acromegaly. Fig. 4. Roentgenogram of skull.

always perspired a great deal but this condition had also become more marked since the onset of the present trouble.

The patient now showed the typical appearance of acromegaly. (fig. 2). Brown pigmented areas were present on the face. Malocclusion of the jaws was present due to protrusion of the lower jaw so that it extended three-fourths of an inch (1.25 cm.) beyond the upper jaw. The tongue was large and thick. His weight which had been 165 lb. (74.8 kg.) was now 230 lb. (104.3 kg.)

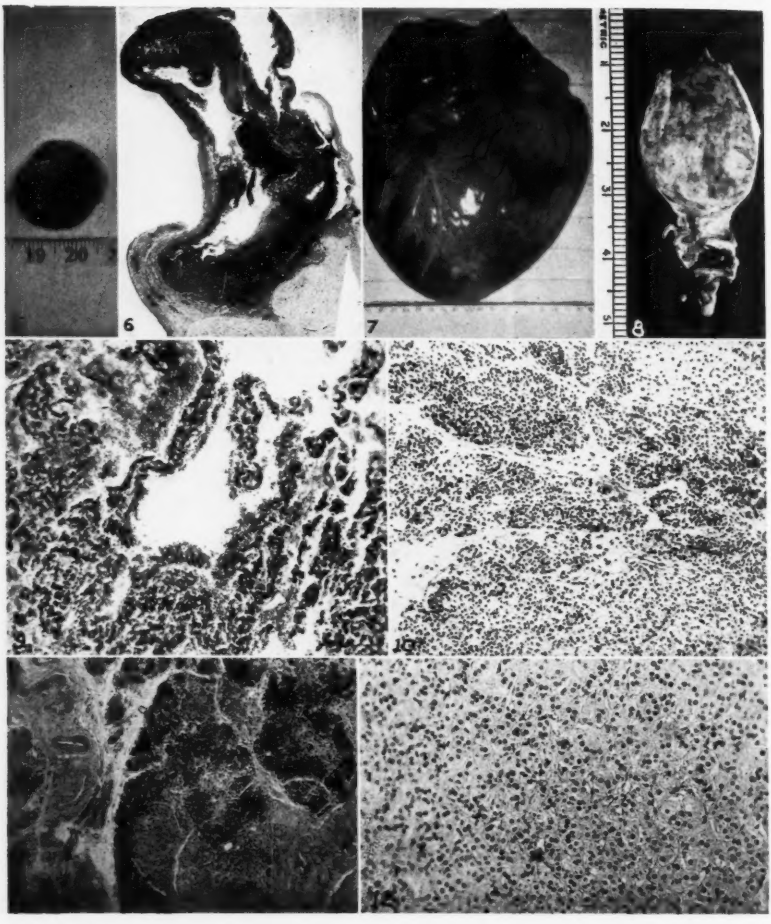

Fig. 5. Pituitary showing enlargement and hemorrhagic condition. Fig. 6. Cross section of pituitary showing cystic, hemorrhagic condition. Fig. 7. Enormously enlarged heart (cor bovinum). Fig. 8. Marked enlargement of right adrenal. Fig. 9. Low power of hemorrhagic cystic area of pituitary. Fig. 10. Section of parathyroid showing solid adenomatous area. Fig. 11. Section of pancreas showing interstitial pancreatitis and adequate insular tissue. Fig. 12. Marked hyperplasia and hypertrophy of right adrenal cortex shown in fig. 8.

A roentgenogram of the skull was reported by Dr. L. Reynolds as a typical acromegalic skull with very large frontal sinuses and protruding lower jaw. The sella was enormously dilated and the floor of the sella completely destroyed in its posterior portion and depressed into the sphenoidal sinus. There was thinning and erosion of the dorsum sella by the enlarged pituitary (fig. 4). A roentgenogram of the left elbow showed decalcification of the bones such as seen in acromegaly.

Findings of perimetric tests and the fundi were normal. The blood pressure was systolic 130, diastolic 90. The Kahn test results and urine findings were negative. The blood count was hemoglobin 85% (Sahli), erythrocytes 4,400,000 per cu. mm.,

leucocytes 5800, polymorphonuclear neutrophiles 54%, small lymphocytes 40%, large lymphocytes 6%.

On December 26, 1928, he came to the office for an acute cold and it was noted that kyphosis of the spine had developed. A roentgenogram of the spine showed a definite decalcification and wedging of the lengthened dorsal vertebrae with resulting kyphosis. The blood calcium was 12.6 mg. per 100 cc. of blood. The only other noticeable feature since the examination in July, 1926, was a change in blood pressure, the systolic being 160, and the diastolic 100.

In May, 1929 he stated that the sight of the right eye had been failing for the last two weeks and that he had frequent temporal headaches. Results of perimetric tests and fundi were reported as normal (Dr. M. Campbell).

The results of two dextrose tolerance tests were as follows.

	First test	Second test
Fasting blood sugar	133 mg.	125 mg.
¾ hr.	180 mg.	200 mg.
1½ hr.	154 mg.	190 mg.
2½ hr.	0.090 mg.	117 mg.

Phenosulfonphthalein test was 15% output in 10 minutes, 45% in 1 hour, and 15% the second hour; or a total of 75% in 2 hours.

During the stay in the hospital his fluid intake and output varied considerably. On one occasion there was an intake of 2700 cc. of water and an output of 1200 cc. of urine. The next day the intake was 1590 cc. and the output 1230 cc. of urine. These figures cannot really be taken as accurate as the patient did not cooperate. Frequent question marks were inserted by the nurses following the intake and output records. The specific gravity of the urine ranged from 1017 to 1020.

He was discharged October 4, and returned October 18, complaining of hematuria of a few days' duration. Cystoscopic catheterization showed that the blood was coming from the right ureter. No calculi or abnormalities of the kidneys were noted in the pylogram. He was discharged October 25, without the hematuria having been accounted for .

The basal metabolic rate was −7%, and −10% four days later. The blood cholesterol was 222 mg., blood nitrogen was 27 mg. The temperature and pulse were normal.

Because of the headaches and the failing vision of the right eye deep roentgen therapy was given over the pituitary area on September 26 and 27.

He was readmitted November 10, 1930, and on November 11, Dr. C. Straith resected about 2 cm. of the symphysis mandibulae, approximating the edges with silver wire. Improvement in the use of his jaws for eating followed the operation and he was discharged. The hematuria had ceased a few days after leaving the hospital and a microscopic examination of the urine revealed a few red blood corpuscles.

In 1931 he went to Los Angeles, California, and entered the General Hospital of Los Angeles County on July 6, 1932. I am indebted to Dr. C. B. Courville of this institution for the report on the patient during his stay there. Excerpts taken from this report follow. Entrance complaint was dyspnea, cough, and swelling of the feet and ankles, duration 2 months. There had been increasing drowsiness for 8 months. The chest was barrel-shaped and there was marked cardiac enlargement. He presented the picture of cardiac decompensation.

Laboratory Data. The urine contained 1+ albumin, dextrose absent, numerous pus cells and a few granular casts in the sediment. The specific gravity was 1024. Daily intake and output were not given. The dextrose tolerance test was essentially

the same as that reported above. The basal metabolic rate was −9% and +18%. A roentgenogram of the heart showed a marked enlargement predominantly of the left ventricle. Electrocardiogram was reported as showing "ventricular extrasystoles; evidence of myocardial damage."

He was given two series of deep roentgen therapy over the pituitary at the California Institute of Technology. He was discharged in August, 1932 and returned to Detroit in March, 1933, entering Harper Hospital on March 25, complaining of extreme dyspnea, weakness, and dizziness.

Outstanding features were marked dyspnea and cyanosis of the lips, ears, fingers and toes. Cheyne-Stokes respirations were present and the patient was very restless. The heart was much enlarged, the apex being near the midaxillary line. Right-sided widening was also present but the borders of the heart were difficult to determine. The heart sounds were almost entirely replaced by a systolic and diastolic crescendo murmur heard over the entire heart area. Many moist râles were heard at the base of both lungs.

The liver was enlarged, the lower edge was felt about 4 cm. below the costal margin. Ascites was present as was marked pitting edema of both legs (fig. 1).

The intake of fluids for 24 hours on the second day after admission was 4260 cc., output of urine 1620 cc. An attempt to restrict the patient's fluid intake made him unruly and he said he would rather die than be deprived of water. Fluids were then given as he desired. Six days before his death the fluid intake dropped to 800 cc. and the urinary output was 240 cc. The specific gravity of the urine ranged from 1018 to 1028. There was 1+ to 3+ albumin present, with many red cells and hyaline casts in the sediment.

An electrocardiogram taken 3 days before death was reported as showing "serious myocardial damage with right ventricular extrasystoles." Decompensation of the heart steadily increased and he died of myocardial failure at 9:50 P.M. April 5, 1933. Autopsy was performed at 11:35 P.M. Measurements of body at autopsy were as follows.

Height	66½ in. (168.5 cm.)
Circumference of head (occipito-glabellar region)	24 in. (61 cm.)
Trans-zygomatic diameter	6½ in. (16.5 cm.)
Trans-occipito-glabellar diameter	8 in. (20 cm.)
Point of chin to vertex of skull	11 in. (28 cm.)
Back of heel to end of great toe	10½ in. (26.5 cm.)
Wrist to tip of middle finger	6¼ in. (16 cm.)
Shoulder to tip of middle finger	30½ in. (77.5 cm.)
Anterior superior spine to sole of foot	39 in. (99 cm.)

On removal, the skull cap was of normal thickness except over the vertex where it was found to be very thin.

The brain weighed 1340 gm. The sella turcica measured 19 mm. from the anterior to the posterior clinoid processes. The pituitary gland was hemorrhagic. In dissecting free the inferior portion of the gland there was an escape of from 2 to 3 cc. of dark red blood. The gland after removal measured 2×2×1.5 cm. (fig. 5) and weighed 2 gm. On section it was found that there was a large hemorrhagic cyst of the anterior lobe measuring about 1 cm. in diameter. The thyroid gland was enlarged.

The tissues of the thoracic wall were edematous. There was approximately 3 liters of light, yellowish-green fluid in the abdominal cavity. The prepyloric region of the greater curvature of the stomach was thickened. On section the stomach wall of this region was about 1 cm. thick.

The liver was markedly enlarged, weighing 2650 gm. The gallbladder was distended but contained no stones. The pancreas was normal. The spleen was slate-

gray in color and weighed 265 gm. A large infarct was present in the lower pole. The right adrenal was markedly enlarged. Its size was 2.5 × 2.5 × 3.5 cm. and weighed 18 gm. It had very much the appearance of an adrenal adenoma (fig. 8). The left adrenal was normal in appearance and weighed 11 gm. The left kidney weighed 240 gm. The right kidney was enlarged, soft and cystic, weight 435 gm. The kidney pelvis and ureter were dilated, the latter being 2 cm. in diameter down to a point on a level with the brim of the pelvis where a mulberry-like stone was found firmly embedded within the ureteral orifice causing an obstruction at this point. On section the kidney pelvis and calyces were found to be dilated.

The aorta showed a few atheromatous plaques. On opening the chest the heart was seen to occupy almost the entire left thoracic cavity causing compression of the left lung. The right lung was congested with numerous adhesions between the diaphragm and the pulmonary base. There was emphysema of all the anterior tissues of the mediastinum. The pericardial sac contained 250 cc. of clear, straw-colored fluid. The heart was greatly enlarged, weighing 1200 gm. a cor bovinum type of heart (fig. 7). The musculature was of normal color and no valvular defects were present but there was a relative insufficiency of the mitral and aortic valves due to dilation of the heart.

The complete anatomic diagnosis was acromegaly, hemorrhagic cyst of the pituitary gland, generalized edema, emphysema of the lungs, pericardial effusion, hypertrophy and dilatation of the heart, ascites, splanchnomegaly, splenomegaly with splenic infarct, enlargement of the right adrenal, right ureteral calculus with hydronephrosis and hydroureter.

The microscopic diagnoses were as follows: colloid hyperplasia of the thyroid gland, marked hypertrophy and hyperplasia of the parathyroid bodies with solid adenomatous areas (fig. 10), adrenal hyperplasia with diffuse hypertrophy of the adrenal cortex (fig. 12), and marked hypertrophy and hyperplasia of the anterior and intermediate lobes of the pituitary gland. In the anterior lobe, the eosinophilic and basophilic tissue has replaced the chromophobe cells. There was also a marked dilatation of blood spaces and in one area these blood spaces have coalesced into a cyst filled with blood (figs. 6, 9).

The pancreas showed localized areas of interstitial pancreatitis with fibrosis but there was adequate insular tissue (fig. 11). The spleen showed passive congestion with hemorrhagic infarct.

The lung showed emphysema with chronic passive congestion. There was moderate pleural thickening.

The testis, bone and bone marrow, spinal cord, aorta, kidney, and stomach were reported as normal. Moderate passive congestion was present in the liver.

The impression of the pathologist, Dr. Plinn Morse, was as follows. "The outstanding microscopical changes consist of pituitary hyperplasia and unilateral adrenal hyperplasia which is greater than either the pituitary change or the marked hypertrophy and hyperplasia of the parathyroids."

COMMENT

The course of the acromegaly from its inception to death was a matter of 8 years. Death was the result of myocardial failure and is therefore similar to cases reported by Courville and Mason (2). These authors report on the observations of 24 patients with acromegaly. Eighteen (75%) presented evidence of heart failure, and 6 died of heart failure. The latter had marked

splanchnomegaly and cardiomegaly. In all of these an eosinophilic pituitary adenoma was observed post mortem.

A history of polydipsia and polyuria was obtained at both Harper Hospital and the Los Angeles County Hospital but the hospital records of the former institution did not show any great variation from normal. It must be said however that there was poor cooperation from the patient during his ambulatory state. When bed-ridden and therefore controlled, a 24-hour fluid intake of 4260 cc. and 1620 cc. urinary output were recorded. Much of the fluid intake was no doubt distributed to the tissues and abdomen, since he was then decompensated. Being bed-ridden the fluid intake and output record was no doubt accurate and was more nearly the real normal intake but the output was disturbed because of the decompensation.

The polydipsia and polyuria which had been present all his life was definitely increased with the onset of acromegaly. It was not, however, a true diabetes insipidus since this condition is characterized by the passage of a large amount of very dilute urine. The specific gravity of the urine of this patient was normal.

Certain factors contributed to the polydipsia and the polyuria. The first was no doubt the pituitary gland and more specifically the anterior lobe. Von Hann (3) showed from the reported analysis of his clinical cases of diabetes insipidus that it developed when the posterior lobe was destroyed, only if there was pars anterior tissue present. He suggested that there was an antagonistic relation between the anterior and posterior lobes, the former secreting a diuretic factor and the latter an antidiuretic.

Fisher, Ingram and Ranson (4) have covered the literature on this subject as well as contributing a large amount of experimental work which has helped to give a better conception of this condition. Their conception of the pathological physiology of diabetes insipidus is stated as follows.

> The supraoptico-hypophyseal system regulates the secretion of the antidiuretic hormone by the neural division of the hypophysis. It should be emphasized that the neural division includes the infundibular stem and median eminence. The interruption of the supraoptico-hypophyseal tracts in the hypothalamus causes the neural division to become atrophic and functionally inactive and leads to a deficiency of the antidiuretic hormone in the organism. Likewise, section of the stem high enough to cut all of it and the median eminence away from the hypothalamus brings about a similar atrophy and deficiency. Extirpation of the neural division in all its parts leads to the same hormonal deficiency by virtue of the fact that it removes the site of formation of the antidiuretic principle. The evidence indicates that the antidiuretic hormone acts mainly on the kidney and prevents the secretion of excessive amounts of urine. The deficiency of this hormone, as a result of the types of damage described, brings about a primary polyuria which is followed by a secondary and compensatory polydipsia. The polyuria which develops can be thought of as representing the resultant of diuretic processes in the body unchecked by the antidiuretic mechanism. These diuretic processes are normally under the control of the pars anterior of the hypophysis and the polyuria may, therefore, be thought of as resulting from an uncompensated activity of this gland. In this sense, then, one may speak of diabetes insipidus as being caused by a disturbance of the equilibrium normally obtaining between the neural division and the pars anterior. It should be emphasized that we are speaking of an uncompensated activity of the pars anterior and not a hyperactivity. The pars anterior can be thought of as exerting its diuretic influence through its general control over metabolism and activity, rather than through a specific diuretic hormone. It should be noted that we support the view that diabetes insipidus is essentially a hormonal disturbance, although the secretion of the antidiuretic hormone is under the nervous control of the hypothalamus. Further, we have been led to conclude that the neural division itself and not the pars intermedia is the site of formation of the antidiuretic principle.

Applying Fisher and associates' concept to the patient herein reported, the polydipsia and polyuria might therefore be understood as a compensated hyperactivity of both the diuretic (anterior lobe) and the antidiuretic (hypothalamico-posterior lobe system) factors. This may be the mechanism by which a necessary increase of water exchange in a large body is cared for.

A second factor is the parathyroid hyperplasia with adenoma formation that was present. As we know, polydipsia and polyuria are frequently associated with hyperparathyroidism. The pathological findings in the parathyroid glands as well as the blood calcium of 12.6 mg. per 100 cc. of blood is suggestive. Unfortunately no studies of the calcium balance were made. In view of the work of Hertz and Kranes (5) and Hoffmann and Anselmino (6) showing the action of pituitary extracts on the parathyroids and blood calcium, the pituitary tumor activity might well be responsible for the parathyroid changes which were present and this in turn would effect the blood calcium.

Furthermore the osteoporosis of the skeleton would be accounted for by such an effect. Of importance in this same connection is the association of nephrolithiasis and hyperparathyroidism (7, 8). The patient had as noted a right sided ureteral calculus.

Some of the clinical aspects associated with Paget's disease, osteoporosis, carbohydrate and calcium disturbances was reported by myself and associates (9). In these articles the close association between carbohydrate and calcium metabolism was pointed out as well as the frequent deposition of calcium in the soft tissues in Paget's disease and osteoporosis. Furthermore, the influence of the pituitary gland function in these diseases was stressed and attention called to the osteoporosis present in acromegaly and the pituitary basophilia of Cushing.

That the ureteral calculus itself acted as an irritant to the kidney is also probable. Of interest is the fact that cystoscopic and roentgen studies made two years' previously did not reveal the presence of a calculus. In view of the calcium disturbance present it is singular that this type of calculus was not detected and no mention of such a finding was made in the report received from the Los Angeles Hospital. That the calculus itself was not a primary factor in the production of the polyuria is evidenced by the history since the hematuria developed late in the course of the disease and, its presence no doubt was a result of the preceding calcium disturbance.

The right sided adrenal cortex hypertrophy and hyperplasia is like that so frequently associated with pituitary hyperfunction and well understood by the pituitary-adrenal cortex physiological relationship.

It is an open question as to what influence deep roentgen therapy had on the production of the hemorrhage in the cystic adenoma of the pituitary gland. As we well know spontaneous hemorrhage in an adenomatous goiter is a not infrequent occurrence and it might well have been that the hemorrhage in the pituitary gland was of this same type. But one cannot discount that roentgen therapy in heavy dosage could be a factor in producing the

hemorrhage. At any rate the future course of the patient with a possible hypofunction of the pituitary as a result of the hemorrhage would have been interesting.

Finally the consideration that because of the reciprocal relationship between the pituitary gland and the testis, the orchitis and epididymitis 6 years preceding the onset of the acromegaly, may largely be discounted because of the time element, and the fact that the testicles were normal on section. However, there is a possibility that there was a gradual diminution in the hormonal interstitial testicular secretion without this being evidenced by objective histological changes in the testes.

SUMMARY

A case of acromegaly, seen from its inception and followed until death 8 years later is reported.

Difficulty in eating due to malocclusion as a result of the lower jaw's protrusion led to a plastic operation on the lower jaw.

Polyuria and polydipsia were a clinical feature and the possibilities of its causes are discussed. Headache and mild disturbance in vision were present at times but results of the perimetric tests and fundi were normal. Noteworthy besides the usual findings of splanchnomegaly, was the enormously enlarged heart, a hemorrhagic cyst in an eosinophilic and basophilic pituitary gland, hypertrophy and hyperplasia with solid adenoma formation of the parathyroid glands, marked enlargement with hypertrophy and hyperplasia of the right adrenal cortex, unilateral (right) hydronephrosis and hydroureter with the presence of a ureteral stone.

Roentgen therapy had been given to the pituitary region. Death was the result of myocardial failure.

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CLINICAL AND LABORATORY NOTES

THE RELATIONSHIP OF MALE SEX HORMONE TO THE LEVEL OF BODILY VIGOR IN SENILITY

R. G. Hoskins, Helen M. Levene and Sylvia Bevin*

The common experience that sexual drive and bodily vigor wane coincidentally has lent plausibility to a widespread belief that the general sedateness of old age is due at least in part to diminution or lack of sex-hormone secretion. This belief is in some degree supported by accounts of invigoration of the elderly by various sex-gland preparations. The classic report of Brown-Sequard on the effects of glycerine extract of testes upon himself exemplifies such reports. In general, however, these reports even when made by sophisticated observers have failed to carry conviction to critical students because of failures to control the element of suggestion. Until recently it has been impossible to set up adequate studies of the problem because of lack of sex-hormone preparations of reliable potency.

That several preparations, natural or synthetic, are of satisfactory potency has now been abundantly proved by animal assays. Clinical reports of the efficacy of such materials in conditions of demonstrated hypogonadism are appearing with increasing frequency. Of particular interest to the present theme is the recent report of Stone[1] that the sexual activity of castrated male rats can be completely restored by testosterone propionate. This evidence proves that the rat is explicitly responsive to that hormone, and that the hormone is specifically effective in replacement therapy.

For the past two years we have been engaged upon a study of vigor in elderly rats, utilizing the self-registering activity cages as described by Richter[2] and Hoskins[3] and their collaborators.† In the experiments to be reported male rats ranging in age from 1 year 5 months to 2 years 1 month were employed. These were in apparent good health for rats of that age range. The diet was Purina dog chow with water ad libitum, a diet upon which our rat colony was shown excellent health and development. Sixteen tests on 9 animals were made. The hormone preparation utilized was testosterone propionate‡ in a sesame-oil menstruum. The dosage ranged from single injections of 10 to 25 mg. In some cases 5 mg., 5 times a week was given. Commonly this was administered by deep intramuscular injections but to control the possibility that this method might in itself have served to depress the muscular activity of the animals, intraperitoneal injections were also used.

Inspection of the graphs served to show to one familiar with the technic that the

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† We are indebted to Dr. Milton O. Lee who designed and supervised the construction of the improved style of activity cages that were used.

‡ We are indebted to Dr. Gregory Stragnell of the Schering Corporation for the testosterone propionate (Oreton).

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hormone was without significant effect. A tabulation was made, however, comparing the activity levels the week preceding the injection with those of the experimental week and the week following. In 7 cases the activity increased, in 8 cases it decreased and in 1 case it was the same. The shifts were all within the limits of habitual variation. Similar studies showed that there was no significant after-effect of the hormone. It appeared therefore, that whatever may be the cause of the depression of the spontaneous activity in elderly rats it is not to be ascribed to lack of testosterone. In several instances the animals used in these tests were proved to be capable of responding to other agents. In one instance a hundredfold increase of activity was registered.

Summary. In 16 experiments on 9 senescent or senile male rats injections of testosterone propionate failed to result in any significant augmentation of muscular activity as shown in self-registering activity cages. It is concluded that the bodily inactivity of the elderly rat is not due to lack of testosterone.

ASSAY OF PINEAL EXTRACTS

PAUL ENGEL*

SOME YEARS AGO the author described[1,2] an assay for pineal extracts, based upon their ability to inhibit the action of gonadotropic hormone. This antagonism of the pineal gland to the hypophysis might serve to explain the inhibitory effect of the epiphysis upon sexual development, as seen in clinical observations and experimental investigations. These results have not been confirmed by Fleischmann and Goldhammer.[3] Also, Viñals[4] and Wade[5] have reported a synergistic effect of pineal extracts on the hypophysis.

Fischer[6] has recently reported upon a new test showing an inhibitory action of

TABLE 1

	Number of mice with opened membrane on day					
	1st	2nd	3rd	4th	5th	6th
6 Controls	0	1	3	5	6	6
6 Mice treated daily with 3γ	0	0	0	1	1	1
6 Controls	0	1	2	4	6	6
6 Mice treated daily with 6γ	0	0	0	1	1	1
6 Controls	0	0	3	5	6	6
6 Mice treated daily with 3γ (another preparation)	0	0	0	0	0	0

the pineal gland, based upon the time of opening of the vaginal membrane in infantile female mice. Normally, with mice 22 days old and weighing 8 gm., it can be predicted that the membrane will rupture spontaneously within 6 days in 90% of the animals. Fischer treated 22-day-old female mice with pineal extracts for 6 days and found that the vaginal membranes remained closed. With less purified extracts the opening of the membrane was advanced rather than inhibited, confirming the results

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of Viñals and Wade. Further purification of pineal extracts Fischer was able to eliminate the 'synergic factor' and obtained purely inhibitory effects on the opening of the vaginal membrane.

The experiment reported here is a repetition of some of those of Fischer, who kindly furnished a supply of his purified extract. As is shown in the accompanying table, daily treatment for 6 days with these extracts inhibited the opening of the vaginal membranes in 5 of 6 mice, in three different experiments.

The smallest quantity, 3γ, of the purified extract sufficient to inhibit the opening of the vaginal membrane is called a mouse unit by Fischer. In an earlier publication the author designated as a rat unit the tenth part of that quantity sufficient to inhibit the action of 10 R.U. of gonadotropic hormone. This unit is greater than the mouse unit of Fischer. The detriment to this test is in its dependence upon the use of a gonadotropic preparation. Fischer's assay is more suitable for practical use. Nevertheless it is believed that the demonstration of two principles in pineal gland extracts, acting oppositely upon sexual development, explains the difference between the author's results and those of Viñals, Wade, and Fleischmann and Goldhammer.

Summary. The results of Fischer are confirmed. The inhibition of the opening of vaginal membrane of infantile mice is considered the best test for pineal extracts. The differences in the results of different investigators are caused by the existence of two hormones in the pineal gland.

THE FRIEDMAN REACTION IN INFANTILE FEMALE RABBITS

PAUL ENGEL AND CARLOS E. MORALES*

THE FRIEDMAN[1] reaction is the only test for pregnancy able to compete with the original method of Aschheim and Zondek. The most certain results are obtained by the modification of Schneider,[2] who uses infantile rabbits instead of adult animals. Sometimes errors occur with adult animals and it is also difficult sometimes to obtain adult rabbits isolated for a 4-week period. Therefore the Schneider modification is considered by some[3] as the best pregnancy reaction.

Rabbits weighing 1200 to 1600 gm. are used for the test. European and North American authors emphasize the fact that the reaction is always negative in rabbits of less than 1000 gm. body weight.

In Montevideo one of us, with Buño,[4] made a series of experiments in rabbits of 600 to 800 gm. weight and observed in these animals the characteristic hemorrhages denoting a positive reaction. In experiments made in Vienna, in collaboration with Fleischmann and Goldhammer,[5] the same results could not be obtained. We injected infantile rabbits of 800 gm. weight with the same quantity (100 R.U.) of the same product ("Luteoantin") and these animals did not show any ripe follicles or hemorrhage.

Again, in Bogota, we have had occasion to observe positive results in 12 very young animals, of 700 to 800 gm. weight, injected with pregnancy urines of different origins (we injected 5 cc. to each animal).

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It seems evident that a certain influence of the tropic or sub-tropic climate exists on the endocrine reaction. The reaction which can not be obtained in Europe or in the United States. in animals of 700 to 800 gm. appears in South America. Zondek[6] too has observed positive reactions in Palestine in much younger rabbits than in Germany. However, the use of animals of less than 1000 gm. of weight for the Friedman reaction is not advised as even in a tropical climate some of these animals do not show the typical reaction. For instance, we have seen a positive reaction in two rabbits of 750 gm. weight injected with pregnancy urine, whereas a third one, of the same age and weight injected with the same quantity of the same urine gave a negative reaction.

Summary. The Friedman reaction in female rabbits of 700 to 800 gm. weight remains negative in Europe, whilst it gives positive results in a tropical or sub-tropical climate.

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CURRENT ENDOCRINE LITERATURE

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ADVANCE ABSTRACTS

BASSLER, A. Three cases of masked hypothyroidism having abdominal symptoms. *To appear in this Journal.*

An occasional case of what looks like acute and sometimes a serious type of gastroenterologic condition is due entirely to hypothyroidism. These cases may present a symptomology in which attention to the thyroid as the fundamental cause of the complaint is masked and may not receive direct attention. Some of them even have been operated upon. Three cases are described with the symptoms present and also the findings that led to the supposition that a thyroid activity, which was below normal, was present and the results on the use of thyroid extract detailed.

BOLING, J. L. AND R. J. BLANDAU. The estrogen-progesterone induction of the mating response in the spayed female rat. *To appear in this Journal.*

Mating responses have been induced in spayed female rats 1) by injections of estrogen alone and 2) by injections of estrogen followed by progesterone. A small injection of estrogen followed after a suitable interval by an injection of progesterone is more effective than a larger quantity of estrogen alone in inducing sexual receptivity in the spayed female rat. With respect to length and intensity, the estrogen-progesterone induced heat resembles that of normal rats more nearly than does the estrogen induced heat. It seems likely, therefore, that in the normal rat, as in the guinea pig, sexual receptivity is caused by the synergistic action of estrogen and progesterone produced in the still unruptured follicles. The rat appears to be more sensitive than the guinea pig to estrogen action and less sensitive to progesterone action.

CHANDLER, S. B., AND J. R. HUGHS. The influence of repeated fasting on the parathyroidectomized albino rat. *To appear in this Journal.*

One hundred and six albino rats from the standard stock colony of Loyola University Medical School were divided into 4 groups and at the ages of 30, 60, 120 and 180 days respectively, 25, 45, 21, and 15 were parathyroidectomized. After one week all the animals in each age group were subjected to a period of 24 hours of enforced fasting. The degree of tetany ranged from slight hypertonicity to generalized convulsions which sometimes resulted in death. Such a procedure was carried out each seventh day for 9 to 15 weeks. The mortality was almost entirely confined to the 30 and 60-day age groups. The morbidity (tetany) was high in the 30-day age group, less in those 60 days of age, very small in the 120 and almost none in animals 180 days of age. To check the completeness of parathyroidectomy all tissue removed was sectioned serially and carefully examined. At the end of the experiment the neck regions of 53 operated, and 15 litter-mate controls, were sectioned as a further check on the completeness of operation. Enforced fasting in the parathyroidectomized albino rat induces tetany in a large proportion of animals less than 60 days of age. If animals are over 60 days in age when operated the tetany is not likely to be severe and very few deaths occur. The degree of tetany decreases in the parathyroidectomized animal when fasted at weekly intervals. The lack of food is considered one reason for the morbidity and mortality.

COPE, O., I. KAPNICK, A. LAMBERT, T. D. PRATT AND M. G. VERLOT. Endocrine function and amylase activity. II. Changes in adrenal cortical function in the dog and rabbit. *To appear in this Journal.*

In adrenal cortical insufficiency in the dog there is a rise in blood serum amylase activity. The level of activity reached in advanced insufficiency is higher than that observed in anterior pituitary insufficiency. The change in serum amylase activity is the most sensitive objective measurement in the blood of cortically insufficient dogs yet determined. In a dog developing insufficiency a significantly high level of activity is reached before any changes in nitrogenous or electrolyte substances of the blood are observed. Both adrenal cortical extract and desoxycorticosterone acetate depress the elevated serum amylase activity of the insufficient dog. The relation of adrenal insufficiency to serum amylase activity in the dog represents the most dramatic evidence so far observed of a part played by the adrenal cortex in carbohydrate metabolism. In adrenalectomized rabbits no rise in serum amylase activity is observed. In half the animals a significant fall took place. It is not concluded that the primary function of the adrenal cortex is control of the amylase system or of carbohydrate metabolism. The amylase changes observed, as well as those of electrolytes reported by others, are presumably secondary to some unsolved primary function of the gland.

COPE, O., I. KAPNICK, A. LAMBERT, T. D. PRATT AND M. G. VERLOT. Endocrine function and amylase activity. III. Further observations of blood serum amylase activity in relation to pituitary, pancreas and thyroid function in the dog and rabbit. *To appear in this Journal.*

In a dog with intact anterior pituitary tissue and permanent diabetes insipidus from ablation of the posterior pituitary lobe and stalk, there was no change in blood serum amylase activity. Loss of the posterior lobe was, therefore, excluded as the source of the rise in amylase activity previously described following total hypophysectomy. Injection of A.P. extract in the normal dog causes a fall in serum amylase activity. The fall in amylase activity in acidosis following pancreatectomy in the dog and the rise to normal with insulin administration is confirmed. In the pancreatectomized, hypophysectomized dog, the amylase activity remains within normal limits. The absence of effect of insulin on the serum amylase of the normal animal is not confirmed. Insulin hypoglycemia, maintained for more than six hours, results in a decrease in activity in dogs and rabbits. If the hypoglycemia is maintained in the dog, the level reached in 36 hours is comparable to that seen in diabetic acidosis. The probable reason for this unexpected result is discussed. Thryoidectomy and the thyroid hormone in the dog and rabbit produce as their only effect a greater variation of activity of the serum amylase system. No consistent variation or tendency was encountered. Removal of the pituitary in the rabbit results in a slight fall in serum amylase activity in contrast to the rise seen in the hypophysectomized dog. The significance of the difference in behavior of the serum amylase systems of the dog and rabbit to loss of anterior pituitary and adrenal cortical hormones is discussed. It is suggested that the various hormones influencing the serum amylase system do so indirectly. It is concluded that those endocrine glands which are intimately related to carbohydrate metabolism affect the serum amylase system.

FLUHMANN, C. F. Comparative studies of gonadotropic hormones. 6. Some effects of long-continued daily injections. *To appear in this Journal.*

The long continued daily injection of small doses of various gonadotropic substances into rats 21 to 23 days of age at the start of the experiment indicates fundamental differences which justify their classification into the three groups of, (1) anterior pituitary, (2) chorionic, and (3) equine gonadtropic hormones. The A.P. extract produced a marked increase in ovarian weight, followed by extensive atrophy of this organ and the appearance of castration cells in the anterior hypophysis. The chorionic hormone also produced increases in ovarian weight with a subsequent regression, but even after one year the ovaries remained larger than normal and the hypophysis did not show post-castration changes. The equine gonadotropic hormone early resulted in an increase of ovarian weight which did not surpass that of normal adult rats even with continued injections, and no abnormality of the anterior hypophysis was observed.

HARTMAN, C. G. Studies on reproduction in the monkey and their bearing on gynecology and anthropology. *To appear in this Journal.*

The following points are discussed. 1) Monkeys and apes are indispensable for the experimental study of menstruation. 2) It is now axiomatic that the menstrual interval in women is 'irregular,' as in monkeys and apes. 3) It now appears that non-ovulatory menstrual cycles, discovered to occur in monkeys, are also found in women. 4) Thus far very few data on time of ovulation in women have been adduced comparable in reliability with the data collected for the rhesus monkey, which has a 'safe period.' 5) Implantation in man and the anthropoids is interstitial, in monkeys superficial. 6) Epithelial proliferation accompanies implantation in monkeys, never in man or anthropoids, where the decidual reaction is also more marked. 7) Intermediacy of the chimpanzee between man and monkey in the phylogenetic

series is also shown in the length of time in which prolan appears in the urine of the pregnant female and in the length of gestation.

HOSKINS, W. H., AND F. C. KOCH. The inhibition of comb growth in cockerels and capons by estrone. *To appear in this Journal.*

The intramuscular injection of 0.5 mg. of estrone daily for 2 or 3 weeks into 10 cockerels and 9 incompletely castrated cockerels produced a temporary shrinkage in the comb with resumed growth as soon as the injections were discontinued. In cockerels 1 to 5 weeks of age treated with this dose of estrone for 1 or 2 weeks, the combs and testes were smaller than those of the controls. The simultaneous administration of 0.5 mg. estrone and 0.1 mg. androsterone to capons produced a comb-growth response equivalent to 0.27 international androgenic unit instead of one unit, as would be expected if estrogens did not diminish the comb-growth response in capons to androgens. Estrone applied to the surface of the comb of the capon was ten times as effective as intramuscularly injected estrone in inhibiting comb-growth response to injected androsterone. The estrone apparently acts directly on the comb and indirectly through the pituitary. This inhibiting action of estrogens emphasizes the necessity of separating urine extracts into androgenic and estrogenic fractions if the androgens are to be applied directly to the comb or in pathological cases where high concentrations of estrogens may be excreted.

HOSKINS, W. H., AND F. C. KOCH. The reciprocal nature of the testis-comb relationship. *To appear in this Journal.*

The daily feeding of 6 to 12 gm. fresh cocks' comb or the equivalent in dry weight to rats 3 to 14 weeks of age for 4 to 13 weeks did not injure the testicles, seminal vesicles, prostate or body weight in studies on 49 experimental and 58 control rats. The rats appeared to thrive on the treatment. The daily injection of alcohol extracts prepared from cocks' comb into 40 experimental rats in a period of 3 to 4 weeks caused a loss in weight of the animal, its gonads, and accessory sex organs. The daily feeding of the alcohol-extracted cocks' comb-tissue had no such effect. The feeding of dried comb-tissue to cockerels (57) had no inhibiting effect on the rate of comb growth or testis development. The feeding of dried cocks' comb to capons did not alter their comb-growth response to the injection of androsterone. If combs are removed from cockerels at 2 to 8 weeks of age, no effects are observed on the testis weights 1 to 3 months after comb removal. However, if the combs are removed at 3 weeks of age and the animals are sacrificed 7 months later, a marked increase in testis weight is observed as compared with the normal controls.

HOUCHIN, O. B., AND C. W. TURNER. A method of assay for the fat metabolism hormone of the anterior pituitary. *To appear in this Journal.*

A study is reported on the use of well nourished female guinea pigs in the biological assay of the fat metabolism hormone using a depression of the plasma fat as the criterion. A group of 34 normal females showed an average plasma fat content of 60.44 mg.%. The livers contained 4.19% fat. Upon injection of A.P. extracts in varying amounts the plasma fat of the guinea pig was depressed to an amount varying with the injection. The maximum depression was reached 6 hours after intraperitoneal injection of the extract. At 10 mg. levels, a depression of 50% in blood fat was produced. Fatty infiltration of the liver was associated with the depression of blood fat. One unit of fat metabolism hormone is defined as the minimum amount of extract which will depress the fat of at least 6 animals, on the average, to about 50% of the normal (normal about 60 mg.%) or to about 30 mg.%. To determine the reproducibility of successive assays at the unit dosage, 6 groups of animals were each injected with 10 mg. of the same extract. Of these only one group exceeded a tolerance of $\pm 10\%$.

JOHNSTON, J. A., AND J. W. MARONEY. Observations on nitrogen and calcium balances as affected by growth and gonadotropic hormones administered for short periods to growing children. *To appear in this Journal.*

There are presented observations on the nitrogen balance, and in some instances the calcium balance, in children of the pre- and post-puberty periods following the administration for short periods of antuitrin-G and antuitrin-S. A variety of responses followed—anabolic, katabolic and negative—which might be explained as being conditioned by (1) the presence or absence of a hormonal defect; (2) the state of the stores as judged by the control balance, and (3) the age of the child. Subjects included several girls with menstrual difficulties at puberty, 4 dwarfs—a cretin, an achondroplastic and two ateleotics, and a number of children who apparently had no endocrine abnormalities. Instances of complete loss of response in the nature of antihormone effect were encountered.

MAGOUN, H. W., C. FISHER AND S. W. RANSON. The neurohypophysis and water exchange in the monkey. *To appear in this Journal.*

The infundibular stem in rhesus monkeys was severed through a subtemporal approach. In

some the section was sufficiently high to pass through the median eminence, i.e., the expanded upper end of the infundibulum which is attached to the hypothalamus. In the chronic state some of the animals were polyuric, others were not. A study of serial sections of the hypophysis and overlying hypothalamus showed that the animals with marked polyuria had complete transections of the median eminence or section of its anterior half alone, while the animals with normal urine outputs had sections of the infundibular pedicle below the median eminence. Volumetric determinations showed that in the cases with marked poluria an average of only 5 to 7% of the total volume of the normal neurohypophysis remained proximal to the section and connected with the hypothalamus. In the cases with normal urine outputs, an average of 12 to 16% of the total volume of the neurohypophysis remained connected with the hypothalamus proximal to the section.

PLATTNER E. B., AND C. I. REED. A study of muscular efficiency in rats injected with anterior pituitary growth factor. *To appear in this Journal.*

White rats were injected with 2 to 6 units of antuitrin-growth daily. Myograms made on isolated gastrocnemii showed that, despite the greater body weight of the treated rats, there was no physiological advantage over the control group from the standpoint of endurance or contractile power except where treppe occurred, in which case there was a slight advantage in that the muscles of treated rats gave slightly higher contractions. This advantage disappeared within less than one minute of stimulation at a rate of 80 per minute.

POMERAT, C. M. Studies on the respiration of the newt. II. Effect of temperature in hypophysectomized, immature, thyroidectomized and pancreatectomized males. *To appear in this Journal.*

Using a modified Warburg respirometer designed to measure simultaneously the O_2 consumption and the CO_2 production of the male newt, *Triturus viridescens*, Raf., values were obtained for hypophysectomized, immature (red-eft), thyroidectomized and pancreatectomized individuals at temperatures ranging from 6° to 28°C. Under the conditions of these experiments the respiration-temperature relationship or relative respiratory rate for normal animals was found in a previous study to yield a coefficient of 17,200. This value remained unaffected following the removal of the major endocrine glands (anterior lobe of the pituitary, thyroid, pancreas and gonad). These findings indicate a stability in the relative velocities of the respiratory links which does not entail a shift in chemical pacemaker despite probable overall changes in absolute metabolic rates as a result of hormonal modifications.

RUBINSTEIN, H. S., A. A. KURLAND AND M. GOODWIN. The somatic growth depressing effect of testosterone propionate. *To appear in this Journal.*

Thirty-two male albino rats of Wistar Institute Strain were divided so that 16 animals received daily (except Sunday) intraperitoneal injections of 1 mg. testosterone propionate (perandren, Ciba) from 26 to 79 days of age, while the other 16 served as uninjected controls. Twenty-four hours after the last injection all animals were killed by bleeding. Body weights taken weekly and body lengths taken at the end of the experiment were computed statistically and comparisons between the two groups were made. It was found that with the dosage used, testosterone propionate proportionately depressed body weight and length to a significant degree.

SELYE, H., AND G. MASSON. The effect of estrogens as modified by adrenal insufficiency. *To appear in this Journal.*

Experiments in the rat indicate that following adrenalectomy, the organism becomes extremely sensitive to the toxic actions of diethylstilboestrol. This substance causes thymus and lymph node involution in the adrenalectomized rat. In these respects, diethylstilboestrol acts in a manner similar to that of naturally occurring estrogens. It appears, therefore, that the actions of this compound do not only simulate the sexual effects of estrogens but also their toxic effects and their actions on the lymphatic system. In these experiments, both diethylstilboestrol and estrone caused uterine estrus in the adrenalectomized rat. This fact and a survey of the relevant literature show that the view of Cramer and Horning, who claim that the presence of the adrenals is essential for the action of estrogens, is not tenable.

TIELOWITZ, R. The colchicine test as a method for determining the time of onset and the duration of action of male active substances. *To appear in this Journal.*

Modification of the test of Loewe and Voss by the use of colchicine permits finer analysis of the effect of male hormones on the seminal vesicles than by the usual methods in which the enlargement of the whole seminal vesicle is observed. One may thus observe the action of a single injection of a male active substance at the beginning, during its course and at the end. The first profuse formation of mitoses in the seminal vesicles of young castrated mice is observed 24 to 25 hours after injection of 150γ of testosterone and 27 to 31 hours after injection of 180γ of testosterone propionate. Their activity is still main-

tained after 58 hours, while doses one-tenth as great develop much weaker activity after this time. In general, testosterone propionate produces the most numerous mitoses and their most uniform distribution. The addition of 'X' susbstance to testosterone increases the activity of the latter.

VIDGOFF, B., R. HILL, R. VEHRS, AND R. KUBIN. Studies on the inhibitory hormone of the testicle. II. Preparation and weight changes in the sex organs of the adult male white rat.

A method is described for the preparation of an extract containing inhibitory hormone from bull testis. Sodium sulfate precipitation of proteins was used. The subcutaneous administration of 1 cc. of extract to adult male rats for a period of 28 to 35 days resulted in changes in the gross appearance and weight of the sex organs of experimental animals as compared with those of the controls. The most marked reduction in weight occurred in the posterior and lateral lobes of the prostate. The seminal vesicles shared this reduction in weight to almost the same extent. The weight reduction of the testicles of experimental animals was less constant. Evidence for these changes is presented in pictures and tables.

WEISER, R. S., AND H. KNOTT. The influence of prolonged etherization, trauma and hemorrhage upon the survival period of adrenalectomized rats. *To appear in this Journal.*

The survival periods of young adrenalectomized white rats (approximately 1 month old) were studied following subjection to the various influences of prolonged etherization, trauma and hemorrhage. Etherization was continued for periods of one hour or more following adrenalectomy, hemorrhage was accomplished by bleeding from the tail and quantitative trauma was produced by crushing the anterior abdominal wall with a strong hemostatic forceps. The results showed that prolonged etherization following bilateral adrenalectomy had no influence on the duration of life of white rats whereas, severe trauma following bilateral adrenalectomy markedly reduced the duration of life of these animals. Adrenalectomized rats were much more susceptible to trauma produced 3 days after adrenalectomy than to trauma produced immediately following adrenalectomy. Traces of adrenal gland which remain following partial adrenalectomy served to protect such rats against the lethal effects of severe trauma and in most cases permitted their indefinite survival. Hemorrhage when combined with moderate trauma markedly reduced the duration of life of adrenalectomized rats. The authors feel that, among experienced operators, such factors as infections, diet, and environmental temperature during both pre-operative and post-operative periods are in the majority of cases more important than the effects of surgery in reducing the life span of adrenalectomized rats.

WELLS, J. A., AND R. R. GREENE. The cortinomimetic activity of various sterols. *To appear in this Journal.*

Progesterone has been shown to be cortinomimetic in the adrenalectomized immature male rat. (40 to 50 gm.) In dosage of 4 mg. per day, it is adequate for maintenance of life. Various other related sterols were incapable of producing such a response. These compounds were: pregnandiol (5 to 10 mg.), pregnandiol crystals (5 to 23 mg.), allopregnandiol (2 to 8 mg.), testosterone propionate (1.25 to 5 mg.), testosterone crystals (2 to 5 mg.), androstenedione (2 to 8 mg.), dehydro-androsterone (3 mg.), estradiol dipropionate (2 to 4 mg.), diethyl stilboestrol (4 mg.), triphenyl ethylene (4 mg.), dihydroxy diphenyl (5 mg.), desoxycholic acid (5 mg.), and dehydrocholic acid (5 mg.).

WILLIAMS, C., D. PHELPS AND J. C. BURCH. The effect of testosterone on experimentally produced endometrial hyperplasia in the guinea pig. *To appear in this Journal.*

The effects of theelin alone, testosterone alone, theelin plus testosterone, and theelin withdrawal upon experimentally produced endometrial hyperplasia in the guinea pig were compared. Each of these procedures produced certain definite modifications in the hyperplastic endometrium. Theelin alone caused a slight intensification of the proliferative reaction. Theelin plus testosterone produced decreased proliferation, a decrease in edema and leucocytes, thin-walled cystic glands and an increase in disintegration of surface tissue. These changes were comparable to those produced by testosterone alone, except for the intensification of disintegration. Neither the changes produced by testosterone plus theelin nor those produced by testosterone alone were comparable to the changes produced by theelin withdrawal.

ADRENALS

STEINFIELD, E., L. N. ETTELSON AND W. A. HODES. Adrenal cortex extract. *J. Lab. and Clin. Med.* 23: 329. 1938.

The authors present a survey of the trends in research since more potent extracts of adrenal cortex have become available, and foresee an extended use of these preparations in a variety of diseases associated with adrenal gland dysfunctions. The bibliography is rather extensive and would seem to offer adequate introduction to the present status of knowledge concerning adrenal cortex physiology.—F. A.

SWINGLE, W. W., W. M. PARKINS, A. R. TAYLOR AND H. W. HAYS. A study of the circulatory failure of adrenal insufficiency and analogous shock-like conditions. *Am. J. Physiol.* 123: 659. 1938.

Evidence is presented from studies on adrenalectomized dogs that the pressor action of the adrenal cortical hormone is independent of the changes in serum electrolytes, hemoconcentration or fluid distribution. The adrenalectomized dog is affected by anything that puts a stress on the circulation. Capillary atony is suggested as the cause of death from adrenal insufficiency.—Lena Lewis.

TIPTON, S. R. The effect of cortin on the electrolyte changes in cat muscle during stimulation and recovery. *Am. J. Physiol.* 124: 322. 1938.

Stimulation of the gastrocnemius muscle of the cat under Dial anaesthesia results in a loss of potassium and magnesium (much smaller than K loss), and a gain of water along with sodium, chloride, and calcium. The loss from the muscles increases somewhat as the stimulation time is increased. With long period of stimulation there may be water lost rather than gained. Intravenous injection of isotonic KCl at a rate too slow to be toxic, increased the loss of K and gain of water. $CaCl_2$ tended to produce effects opposite to the KCl. Cortin given subcutaneously in large doses for 3 days prior to the experiment resulted in a decrease in the magnitude of the electrolyte changes of the normal muscle; in other words, a result resembling that of Ca.—F. A. H.

VERZÀR, F., H. HÜBNER AND L. LASZT. Lactoflavin combined with phosphoric acid after adrenalectomy (Die Bindung des Lactoflavins als Lactoflavinphosphorsäure im Körper nach Nebennierenexstirpation). *Biochem. Ztschr.* 292: 152. 1937.

The liver of normal rats contains approximately 0.001% total flavin, approximately 5% of which is free, and the remainder combined as yellow enzyme. The total flavin is reduced by approximately 50% during the first 4 days following adrenalectomy in rats, cats, and dogs, the amounts of free and combined flavin becoming approximately equal.—*Physiol. Abst.*

v. ISSEKUTZ, B. JR., L. LASZT AND F. VERZÀR. Derangement of the absorption of sugar and fat after adrenalectomy (Zucker und Fett-Resorptionsstörungen nach Nebennierenexstirpation). *Arch. f. d. ges. Physiol.* 240: 612. 1938.

Normal cats absorb glucose twice as fast from the gut as they do xylose. After adrenalectomy the rate of absorption of these sugars becomes equal while the rate of absorption of fats is greatly diminished.—A. G.

v. ISSEKUTZ, B., JR., AND F. VERZÀR. The rôle of the anterior pituitary and the adrenal in the migration of fat (Die Rolle des Hypophysenvorderlappens und der Nebenniere bei der Fetwanderung). *Arch. f.d. ges. Physiol.* 240: 624. 1938.

CCl_4 or P poisoning fail to induce fatty infiltration of the liver in hypophysectomized rats. The administration of cortin restores the normal deposition of fat.—A. G.

GONADS

ENGE, L. A., K. M. MURPHY AND W. SCHILLING. Effect of theelin on transplantable mammary rat adenofibroma. *Proc. Soc. Exper. Biol. & Med.* 38: 21. 1938.

Thirty-two male and female rats ranging from 135 to 150 days of age were implanted with fast-growing mammary adenofibroma of the strain 5-B1. These animals were divided into 3 groups; the first group, consisting of 6 females and 5 males, were given 100 I.U. of theelin; and the second similar group with 200 I.U. over a period of 77 days; the third group of 5 males and 5 females as controls.
The successful tumor transplants recovered from male recipients grew more glandular tissue than those recovered from the females of all groups. There was no difference, however, in the extent of glandular development in the tumor tissue from the male and female rats treated with theelin from those of the corresponding controls. In general no demonstrable growth effect was produced in the transplanted tumor tissue following long continued theelin treatment beyond that expected from the normal mammary gland tissue. In fact, the transplanted tumor tissue seemed to have lost the greater part of its adenomatous components.—E. T. Gomez.

ENGEL, P., AND E. NAVRATIL. On the destruction of the follicular hormone in cold blooded animals (Beitrag zur Zerstörung des Follikelhormons beim kaltblüter). *Biochem. Ztschr.* 292: 434. 1937.

Confirming Zondek, liver-brei inactivated follicular hormone, whereas muscle-brei had no effect. The hormone was not destroyed by perfusing it through the liver. If injected into the lymph sac almost complete inactivation occurred within 48 hours; the result was the same in normal and hepatectomized animals. It is concluded that the inactivation of the hormone does not occur in the liver exclusively.—E. Bozler.

ENTENMAN, C., F. W. LORENZ AND I. L. CHAIKOFF. The endocrine control of lipid metabolism in the bird. 1. The effects of pregnant mare serum upon the blood and liver lipids of the domestic fowl. *J. Biol. Chem.* 126: 133. 1938.

The daily injection of 150 R.U. of gonadotropic hormone in pregnant mare's serum for periods varying between 8 and 27 days produced a highly significant rise in the whole blood lipids of 18 immature female fowl as compared with 14 control birds. These changes were similar to those observed in mature laying birds. It was shown that the formation of yolks was not the stimulus for the rise in blood lipids. A relationship was shown between the rise in blood lipids and oviduct size. The livers of the injected birds showed normal amounts of all lipid constituents.—L. O. Randall.

FRADA, G. Functional relationship in pregnancy between hypophysis and epiphysis and their repercussion on carbohydrate metabolism (Correlazioni funzionali in gravidanza tra ipofisi ed ipofisi e loro repercussione sul ricambio idrocarbonato). *Biochim. e terap. sper.* 25: 417. 1938.

Intramuscular injections of an aqueous suspension of a chloroform extract from 0.2 gm. pineal gland powder caused a moderate increase in the blood sugar of the male or non-pregnant female rabbit. Pregnant rabbits showed a decline or no effect. An aqueous extract produced a slight decrease in normal, a slight increase in pregnant animals. Since an analogous reaction on sugar increase was obtained with AP extract, it is concluded that the pineal gland acts by way of the pituitary.—A. E. M.

FRIEDMAN, M. H. Gonadotropic extracts from the leaves of young oat plants. *Proc. Soc. Exper. Biol. & Med.* 37: 645. 1938.

Oat plants, cut 4 to 6 inches high and either sun cured or rapidly dehydrated when extracted by a modification of the Katzman-Doisy benzoic acid procedure yielded a product having gonadotropic activity. A single intravenous injection of this product in amounts equivalent to 30 to 80 gm. of the original dry material into rabbits produces ovulation in less than 18 hours.—E. T. Gomez.

GAVIOLI, R. L. The antilactogenic action of folliculin (Acción antigalactógena de la foliculina). *Semana méd.* 45, II:130. 1938.

From 45 treated cases and 24 controls the following conclusions are obtained: Folliculin is efficacious in reducing or abolishing the milk secretion after delivery. The dosage should be high and given soon after expulsion of the placenta. With 50,000 R.U. of dihydrofolliculin benzoate, given in a single injection, 83% favorable results were obtained. The relief of congestion of the breasts was conspicuous.—A. E. M.

GREENE, R. R., M. W. BURRILL AND A. C. IVY. The experimental production of intersexuality in the female rat. *Am. J. Obst. & Gynec.* 36: 1038. 1938.

By injecting into pregnant rats testosterone in varying degrees almost two thirds were caused to abort or resorb the young. Of the rest, the females in 86% showed some evidences of masculinization. Details are presented to illustrate the varied pictures found.—E. L. S.

GROHER, H. The effect of male gonadal hormone on infantile ovaries (Die Einwirkung des männlichen Keimdrüsenhormons auf infantile Ovarien). *Arch. f. Gynäk.* 165: 347. 1938.

Ten-day-old mice, 12 test animals, and 12 controls were used. A steer testis extract called "testiglandol' in which 1 cc. is equivalent to 4 gm. of fresh tissue was used. Mice received from 1.2 to 4.5 cc. of extract in all and were killed on the 18th to the 40th days. Fewer and more atretic follicles were found in test animals. Changes are attributed to a depressing effect of male hormone on the sex center in the midbrain which governs release of gonadotropic hormones.—F. A.

GRUMBRECHT, P., AND A. LOESER. The rôle of the uterus in the action of the ovarian hormone on the thyroid (Die Funktion des Uterus bei der Wirkung der Ovarialhormone auf die Schilddrüse). *Arch. f. Gynäk.* 167: 199. 1938.

The authors first point out that in oöphorectomized rats the thyroid gland shows enlargement of the follicles, flattening of the follicle epithelium, and increase in amount of colloid. They state that this condition is associated with an increased amount of thyrotropic hormone production and a decreased response of the thyroid to this hormone. The new observations with which their paper is concerned are that the same thyroid changes can be produced by extirpation of the uterus, by tying of the tubes, or by opening the peritoneal sac with which the rat's ovary is surrounded. It is their belief that in the rat ovarian hormones get in the blood not only by the ordinary route, but by way of the ovarian peritoneal sac from which they make their way down the tubes into the uterus. The authors also point out that the castration effects on the thyroid gland cannot be rectified by estrin therapy. They believe that in order for estrin to prevent the thyroid changes it must pass through the uterus. They further showed that intrauterine injections of estrin in castrated rats prevented the thyroid changes. They suggest 3 hypotheses: *a*) that estrogen is chemically changed in the uterus and becomes thyrotropic; *b*) that estrin

stimulates the uterus to produce thyrotropic substances; or *c*) that estrin after it passes through the uterus makes the thyroid more susceptible to thyrotropic hormone.—F. A.

GRUMBRECHT, P., AND A. LOESER. Proliferation of endometrium by the intrauterine action of estrogenic substances (Aufbau der Uterusschleimhaut bein intrauteriner Einwirkung von Oestrogenen Substanzen). *Arch. f. Gynäk.* 167: 373. 1938.

Animal experiments were carried out on guinea pigs. These showed uterine administration of estrone, estradiol, or estradiol benzoate, caused proliferation of the endometrium and hypertrophy of the uterine muscle. Much smaller doses were necessary than by the subcutaneous route. Furthermore the proliferation occurred much more rapidly (within 24 hours) and lasted longer. The author also introduced estrin into human uteri by means of oleum cacao suppositories, each suppository containing 500 I.U. Here again marked proliferation of the endometrium was obtained.—F. A.

GULDBERG, E. Real menstruation in a castrated woman after administration of synthetic hormones. (Echte Menstruation bei einer kastrierten Frau nach Zufuhr von synthetisch hergestellten Hormonstoffen). *Zentralbl. f. Gynäk.* 62: 2584. 1938.

A 22-year-old woman who had bilateral salpingo-oöphorectomy had climacteric symptoms for 10 months. She was treated with the synthetic diethylstilbestrol intramuscularly in oil, 20.25 mg. in 18 days, followed by 30 RB. U. of synthetic progesterone in 6 days. The uterus grew larger, endometrium changed from castrate type to cystic glandular hyperplasia, and finally to some evidence of progestational glands, flow followed during the progesterone therapy, the climacteric symptoms were controlled, breasts hypertrophied, and there was some evidence of reduced excretion of urinary gonadotropic hormone during therapy. Oral use of the estrin was shown to control symptoms and produce the typical estrin reaction in the endometrium.—E. L. S.

HAIN, A. M. Oestrogenic and androgenic substances in pregnancy. *Edinburgh M. J.* 45: 678. 1938.

The urine of 45 pregnant women was examined by carefully standardized and controlled methods. In assaying the female sex hormone, special care was used to separate the relatively inactive form of the hormone, estriol, from the more active estrone. The studies were confined largely to the last 2 months of pregnancy. The author concludes: *a*) that marked differences in hormone concentration exist between individuals; therefore cases should be considered individually. *b*) That the data show the trend of hormone output during the latter part of gestation, but that it is advisable to make more frequent estimations of the hormone concentration than at monthly intervals, and that, during the last 10 days, daily estimations would be likely to give valuable information. As it is, it would seem that the upward trend in hormone concentration which occurs in the last month of pregnancy is preceded by a relatively low level. *c*) That there is no indication that very high values of estrin are necessarily associated with labor either in the 'combined' or 'free' form. *d*) That by far the greater portion of the estrogenic substance excreted in pregnancy is found in the form of estriol. *e*) That the androgens do not bear any quantitative relationship to the estrogens, but show a tendency to share in the general hormone decrease at the 9th month and in the rise during the last month of gestation. *f*) There is less androgenic substance excreted by the pregnant than by the non-pregnant woman.—J. C. D.

HYPOPHYSIS

BURKE, J. C., AND A. R. MCINTYRE. The effects of vitamin B on insulin hypoglucemia and sugar tolerance. *J. Pharmacol. & Exper. Therap.* 64: 465. 1938.

Glucose and insulin tolerances were determined on albino rats, 125 to 350 gm. in weight, kept on a vitamin-B-free basal diet. Vitamin B_1 increases the hypoglucemic response to insulin. Autoclaved, flavin-free rice polishings concentrate decreases the hypoglucemic effect of insulin.—E. L.

BUTT, H. R., AND A. KEYS. Effect of large doses of insulin on the proteins and the colloid osmotic pressure blood serum. *Arch. Int. Med.* 63: 156. 1939.

The effect of injections of insulin on the protein content and the colloid osmotic pressure of the blood serum was studied in physically normal men with schizophrenia and in dogs. Doses of 1 U of insulin per kg. of body weight were used for the dogs and doses of 20 to 130 U for the men. In all cases after the administration of insulin there was a rise in the concentration of total serum protein; in 19 studies on men this increase averaged 4.7%. On the average the albumin concentration changed in proportion to the total protein content. In all cases there was an increase in the colloid osmotic pressure of the serum of the men; the average rise for 9 studies was 9.7%. Calculations are presented to show that on the average the mean molecular weight of the serum protein is unchanged in acute insulin hypoglycemia. It is concluded that 'insulin shock' bears no close relation to other types of shock, that the total blood volume

is at most only slightly altered and that the principal change in the blood concentration is a result of the addition of new red blood cells to the active circulation.—I. B.

DUFF, J., AND F. W. WILLIAMS. Diabetics in surgical urology. *J. Urology* 40: 446. 1938.

Among 3158 urologic patients examined in a 5-year period, there were 1.3% of diabetics which is about the rate found in males in general. Of these 39 diabetics, 17 were operated on. Their medical care was placed in the hands of a special 'Internist attached to Surgery for Diabetes.' The authors feel that the dangers of surgery in diabetics can be so minimized that they are scarcely greater than those in non-diabetics. They conclude: "The urologic patient whose case is complicated by diabetes should be cared for by a urologist together with an internist where there is good organization for diet supply. Dehydration and severe ketosis are the only contraindications to urgent surgery. Absolutely sugar free urine and low blood sugar are not essential before operation. The type of diabetes encountered in urology is usually mild and the deaths should not be of diabetic origin. Insulin should be used cautiously, mainly for the purpose of combating dehydration, and ketosis and controlling severe glycosuria. Insulin if pushed to sugar free urine and low blood sugar may cause dangerous hypoglycemia.—J. C. D.

DUNLOP, D. M. Zinc protamine insulin. *Edinburgh, M. J.* 45. *Tr. Med-Chirurgical Soc.* 194. 1938.

The report is based on 150 patients, 102 of whom had had sufficient experience with soluble insulin to make comparison with the new type valuable. Larger doses of Z. P. insulin were required in 25 of these cases, smaller in 63, and identical amounts in 14. One case could not tolerate the Z. P. insulin and 4 additional patients went back to the soluble type for various reasons. Supplementary doses of soluble insulin were necessary in only 25 cases.—J. C. D.

GRAYZEL, H. G., AND L. S. RADWIN. Hepatomegaly in juvenile diabetes mellitus treated with pancreatic extract. *Am. J. Dis. Child.* 56: 22. 1938.

The authors state that in 3 young diabetics with persistent hepatomegaly, striking recession of the liver to its normal size was demonstrated only after the addition of specially prepared pancreatic extract. Adequate management of the diabetic condition with dietary control and insulin therapy alone had failed to accomplish this result. The pancreatic preparation must be continuously administered to prevent recurrence of hepatomegaly. The dramatic recession of hepatomegaly and the marked lowering of the level of the blood lipids following administration of the extract justify the tentative assumption that the enlargement of the liver was due to fatty infiltration and that the therapeutic agent is a lipotropic substance.—M. B. G.

LASCH, F. Ultrafiltration tests with insulin (Ultrafiltrationsversuche mit Insulin). *Biochem. Ztschr.* 297: 244. 1938.

A fraction of the commercial insulin preparations tested passed through ultrafilters which held back proteins. The filtrate was physiologically active but the potency was 5 to 10 times smaller than that of the original preparation. If the filtrate was concentrated, crystals were formed which, however, had no effect on blood sugar.—E. Bozler.

NEWTON, W. H., AND F. G. YOUNG. The influence of the glycotropic (anti-insulin) factor of the anterior hypophysis on the insulin sensitivity of the hypophysectomized rabbit. *J. Physiol.* 94: 40. 1938.

It was found that 0.1 U insulin injected into a hypophysectomized rabbit after 2½ hr. of fasting produced the same fall in blood sugar as did 2 U in an intact rabbit fasting 21 hr. Seven hypophysectomized rabbits were given 2 injections of glycotropic factor (which is contained in prolactin) equivalent to a total of about 13 gm. of fresh anterior lobe, then fasted for 2½ hr. and given 0.1 U of insulin. The hypoglycemic action of insulin was diminished but not to the same extent that had been previously shown to occur in normal animals.—J. W. Jailer.

ROBINSON, G. W., JR. Insulin therapy in acute alcoholic psychosis: Study of nine successive cases of alcoholic psychoses treated with insulin. *J. Kansas M. Soc.* 38: 463. 1937.

Shortly after beginning the use of insulin in treatment of the withdrawal symptoms of alcoholism Robinson encountered a case of acute hallucinosis with insomnia, delusions, hallucinations, complete disorientation and occasional attacks of extreme, serious mania. Seven hours after the patient's admission to the hospital and 1½ hours after the second dose of 20 U of insulin, these symptoms had cleared completely. After this 9 patients with acute alcoholic psychoses were treated with insulin. From 40 to 50 U divided into 2 doses was effective in cases of uncomplicated psychosis, but the amount of insulin indicated in an individual case was that sufficient to produce the desired results. An uncomplicated psychosis responded to the following technic: twenty U of insulin is given subcutaneously immediately on admission. During the next 3 hours the patient drinks all the orange juice that can be forced. The second injection of 20 U is given 3 hours after the first. The minimal requirement of orange juice to prevent

reaction from the second dose is at least 30 ounces (900 cc.). Ordinarily, the first dose will have no effect on the patient other than to produce hunger and, therefore, cooperation in the taking of fluids and nourishment. The mental symptoms usually are not improved until after the second injection. From ½ to 1 hour after the second dose the patient begins to quiet and soon falls asleep. Sleep may be fitful for a few hours, but from 3 to 5 hours after the second dose the patient falls into a deep sleep, from which he awakens clear. The psychosis may or may not return after this sleep. A small dose of insulin is given shortly after the patient awakens, and thereafter no more is used unless the psychosis returns or the patient is not completely clear. If further injection of insulin is indicated, at least 4 hours should elapse between the awakening and the resumption of treatment, during which carbohydrates are forced by every possible means, and the routine of administering insulin is repeated at the end of this period. Six of the author's patients responded perfectly to the treatment. The other 3 patients had complications, 2 an infection of the upper part of the respiratory tract and the other syphilis.—From abstract in *Arch. Neurol. & Psychiat.*

SANTENOISE, D., C. FRANK, R. GRANDPIERRE AND M. VIDACOVITCH. Effect of pancreatectomy on the number of red corpuscles in the blood (Effet de l'ablation du pancréas sur la teneur du sang en hématies). *Compt. rend. Soc. de biol.* 126: 354. 1937.

From experiments on more than 25 dogs, the authors conclude that pancreatectomy results in a progressive anemia, which is not relieved by insulin. Simultaneous administration of vagotonin will prevent it or, if the anemia is already established, will relieve it. The pancreas, therefore, exercises through the action of vagotonin a marked influence on the number of red corpuscles.—J. C. D.

WATSON, B. A. Normal glucose tolerance tests. *J. Lab. and Clin. Med.* 23: 364. 1938.

A statistical study of blood sugar levels in 83 normal young adults is presented. The average daily diet consisted of carbohydrate 300 to 400 gm.; protein 60 to 90 gm.; fat 90 to 120 gm. and blood sugars (micro method) were taken at 0. 30, 60, 120 and 180 min. after receiving 100 gm. glucose by mouth. A high incidence of glycosuria (10.8%) in these subjects was found. Twenty cases of young obese adults showed practically the same findings as did normal weight groups. Subjects with family history of diabetes showed no evidence of diminished carbohydrate tolerance.—F. A.

WHITE, P. Endocrine manifestations in juvenile diabetes. *Arch. Int. Med.* 63: 39. 1939.

The juvenile diabetic patient shows evidence of a disturbed general hormonal balance. There is striking evidence of hyperactivity of the pituitary body in the prediabetic stage, followed by diminution of activity which in its most extreme form occurs in the diabetic dwarf. Some forms of endocrine disturbance are presumably accidental, namely, those related to the thyroid gland. Although the evidence does not exclude primary insulin insufficiency and although no form of therapy in diabetes yet compares with insulin therapy, this interrelation of the endocrine glands gives the clue for future research. At present investigators throughout the world are searching either for an antihormone other than insulin or for the pancreatropic factor.—I. B.

WOHL, M. G. Spontaneous hypoglycemia response to insulin therapy. *Internat. Clin.* 4: 180. 1938.

Three case histories illustrate this paper on spontaneous hypoglycemia. In 1 case the symptoms disappeared as the result of a high fat, low carbohydrate diet and insulin administered after meals. In the second case the addition of thyroid extract to insulin treatment controlled the symptoms. The third case was partially controlled by insulin. The disturbed carbohydrate metabolism responsible for spontaneous hypoglycemia may be pancreatic or nonpancreatic in origin. Successful treatment depends upon proper diagnosis. Etiological discrimination is at times a difficult problem. Addison's disease may simulate hyperinsuliniam.—I. B.

PANCREAS

MIKLOS, L. New observations on the action of the gonadotropic hormone (Neue Beitrage zur Wirkung des gonadotropen Hormons). *Zentralbl. f. Gynäk.* 62: 2745. 1938.

The rabbit uterus was brought to a state of marked development with stimulation by intravenous administration of urine from a pregnant woman, then after the animal was castrated treatment was carried on with commercial luteoantin, which the authors had tested and found to be devoid of estrin action in the castrate mouse. This preparation was able to maintain for several days the developed state of the uterus, whereas controls not treated after castration showed rapid atrophy of the endometrium. Castrated mice were treated with commercial estrin, glandubolin, and then the estrin development continued, as judged by the gross appearance of the uteri, when only the gonadotropic material was used. The results are thought to demonstrate that the gonadotropic material has a direct effect upon the

uterus after it has been brought to a high state of development by estrin or estrin and progestin. (There is no statement as to whether the gonadotropic material is APL or whether any progestational activity was included in it.)—E. L. S.

MURPHY, R., S. LOWTHER AND L. PAGNIELLO. Organ hypertrophy following injections of thyreotropic hormone. *Am. J. Physiol.* 124: 110. 1938.

Experiments have been reported which indicate that in spite of the refractoriness of the rat to thyrotropic hormone a slight increase in the size of the heart and a greater increase in the size of the kidney occur when sufficiently large doses of a fairly pure thyrotropic extract are administered. The response of the thyroid and kidney can be considerably enhanced by 'dye-blocking' the reticulo-endothelial tissue previous to and during the period of injection. The results suggest that an inhibitory substance is present in the rat previous to the 10th day of administration of thyrotropic preparations.—Authors' Summary.

NECHELES, H., AND F. NEUWELT. Antagonism between posterior pituitary secretion and acetylcholine. *Am. J. Physiol.* 124: 142. 1938.

Pitressin, but not oxytocin, antagonizes the depressor effects of small doses of acetylcholine in dogs. Pitressin also inhibits salivary secretion and has an effect on the vagal inhibition of the heart, probably secondary to the ischemia produced by the hormone. Even the small amounts of pitressin liberated reflexly from the pituitary by central vagus stimulation (Lim et al.) effectively antagonize acetylcholine, suggesting that this antagonism represents a physiological mechanism.—E. Bozler.

PESME, P., AND G. HIRTZ. Adiposogenital syndrome and pigmentary retinitis (syndrome of Bardet-Laurence-Biedl). *Rev. d'oto-neur-opht.* 15: 257. 1937.

The case reported is that of a boy aged 8½ years with an adiposogenital syndrome, visual acuity of 1/10 in each eye and pigmentary retinitis. The patient's mother was obese and had signs of insufficiency of the anterior lobe of hypophysis and sympathicotonia. The patient weighed 40 kg. and was 130 cm. in height. Under treatment with extract of the anterior lobe of the hypophysis and with thyroid, adrenal and whole pituitary, followed by the administration of extract of embryonal tissues, a marked improvement in the condition occurred. There were no signs of malformation of the skeletal system or the sella turcica. The sugar tolerance was normal. There was no polyuria, lymphocytosis or disturbance in thermogenesis or metabolism.—From abstract in *Arch. Neurol. & Psychiat.*

PESME, P., AND G. HIRTZ. Blindness of hypophyseal origin: Four cases of the adiposogenital syndrome with retinal degeneration and mental backwardness. *Gaz. méd. de France* 44: 833. 1937.

Pesme and Hirtz recently studied 4 cases of a rare hereditary and familial syndrome that is characterized by adiposogenital dystrophy with mental backwardness and retinal degeneration. The condition differs from that of the Laurence-Biedl syndrome in the absence of malformations of the members (syndactylism or polydactylism). Three symptoms characterize the typical as well as the atypical forms: obesity of the hypophyseal type, mental backwardness and retinal disorders. The retinal symptoms are especially noteworthy: In all cases there was degeneration of the retina with discoloration and hemeralopia. One of the patients was given extracts of the entire hypophysis for a year without the slightest improvement. Then extracts of the anterior lobe of the hypophysis and with several other glandular extracts (thymus, thyroid, adrenals and total hypophysis) were used. The patient lost weight and increased in height; the genitalia developed to normal size; the hands and fingers, which had been extremely short, assumed normal shape, and the nails lost their friability. The boy became more active and bright. Even more remarkable was the change in the ocular defects. The boy was able to fix his vision without being disturbed by nystagmus, and the photophobia disappeared. Measurement of the visual acuity showed hardly any increase, but the boy was better able to utilize his visual powers. On the basis of this observation, the authors conclude that the hypophysis influences not only the fat metabolism and the development of the genital organs but also the retina. The authors think that treatment with hypophyseal hormones may prove valuable in some congenital retinal defects.—From abstract in *Arch. Neurol. & Psychiat.*

RICHARDSON, H. B. Simmonds' disease and anorexia nervosa. *Arch. Int. Med.* 63: 1. 1939.

This report concerns 6 patients, 3 of whom were thought at one time to have pituitary cachexia, or Simmonds' disease, and all of whom proved to have anorexia nervosa. Apart from the psychic aspects, the symptoms, physical signs and laboratory findings differed in no essential from those reported in the literature in cases of Simmonds' disease in which the diagnosis was proved at autopsy. In both diseases the manifestations are those of inanition. In the diagnosis of a destructive lesion of the anterior lobe of the pituitary body when there is no evidence of an expanding lesion of the anterior lobe, the chief clues are the age and the number of pregnancies. The diagnosis of Simmonds' disease should be withheld until persistent efforts have been made to demonstrate a neurosis and to relieve it if present or until

attempts have been made to increase the intake of food and vitamins. Even then the diagnosis may remain in doubt. Replacement therapy in cases of the type of condition often diagnosed as Simmonds' disease rests on an extremely insecure basis. There is no conclusive demonstration of the action of the extract of the anterior lobe, either in proved cases of Simmonds' disease or in pituitary dwarfs who have passed the normal age of puberty. In the treatment of patients who had anorexia nervosa, no physiologic effect of the extract of the anterior lobe could be demonstrated. The doses were small. Insulin was used in case 1 and caused a powerful stimulation of the appetite. In case 3 it was received without benefit. The administration of thyroid in case 4 was accompanied by an alarming acceleration in the loss of weight. An adequate intake of vitamins is of obvious importance. Coercive measures designed to increase the intake of food are dangerous. Such attempts are directed at the presenting symptom rather than at the cause. The treatment is essentially that of the neurosis. This should not imply neglect of the purely medical aspects of the case.—I. B.

Soskin, S., R. Levine and R. E. Heller. Carbohydrate utilization in the hypophysectomized dog. *Proc. Soc. Exper. Biol. & Med.* 38: 6. 1938.

The rate of utilization of carbohydrate was determined under various blood sugar levels of dogs eviscerated and hypophysectomized 4 to 8 weeks prior to the initiation of the experiment. The experiments showed that hypophysectomized dogs utilized markedly less sugar at any given blood sugar level than normals and much less so than those of depancreatized dogs at the blood sugar level ordinarily maintained by the diabetic animals (350 mg. per cent). The average hourly decrease of muscle glycogen or the average hourly increase of lactic acid level was definitely less than those of either the normal or depancreatized dogs. This observation was attributed to the relative stability of muscle glycogen. In general the above observation was interpreted as indicating that in dogs the rate of utilization of carbohydrate is greatly diminished following complete removal of the hypophysis.—E. T. Gomez.

PARATHYROID

Hanes, F. M. Hyperparathyroidism due to parathyroid adenoma, with death from parathormone intoxication. *Am. J. M. Sc.* 197: 85. 1939.

An instance of hyperparathyroidism with fatal parathormone intoxication is reported, thus filling an existing gap in the natural history of the disease. A characteristic type of parenchymatous renal calcification is illustrated, which is believed to be pathognomonic of hyperparathyroidism. A blood calcium level approaching 20 mg.% is indicative of severe and dangerous parathormone intoxication.—Author's Summary

Leriche, R., and A. Jung. A chemical and therapeutic study on tetany (Recherches chimiques et thérapeutiques sur la tétanie). *Presse méd.* 46: 897. 1938.

Tetany may be spontaneous or following parathyroidectomy. The blood calcium is always low in the latter, but not necessarily in the former type. In 4 cases of spontaneous tetany the total urinary Ca per liter of 24 hours was below 0.045, the fecal Ca normal and the Ca balance positive. Calcium gluconate raised the urinary Ca only insignificantly. Certain types of spontaneous tetany were not relieved by parathyroid hormone. Transplanation of bone under the skin failed, in three cases, to influence the blood Ca, increased the urinary output only slightly but suppressed the tetanic crisis, a relief lasting in one case for 1½ years. Middle cervical sympathectomy or enervation of the carotid sinus with the object of activating the parathyroids cured the tetany in 5 cases although the blood Ca was not influenced. It is concluded that tetany is caused by parathyroid deficiency with defective utilization of the Ca reserves. As treatment of spontaneous tetany is recommended: transplantation of bone and sympathectomy.—A. E. Meyer.

Pillsbury, D. M., and T. H. Sternberg. Lichen urticarius (popular urticaria); treatment with parathyroid extract, theoretical consideration of the etiology. *Am. J. Dis. Child.* 53: 1209. 1937.

Parathyroid extract was given subcutaneously in doses of from 10 to 25 U at weekly or bi-weekly intervals to 34 of 60 patients with lichen urticarius. All were given calcium internally and all but 11 had some topical application. The results indicate that this is an effective method of treatment, the response being good or excellent in 85% of the patients as compared with 38% in the control group. The action of parathyroid extract is the elevation of the Ca content of the blood. The favorable influence of this extract in this disease may be explained either as due to the sedative effect of an increased diffusible fraction of the blood Ca or as a partial or complete correction of some underlying fault in the Ca metabolism. The literature is reviewed.—M. B. G.

THYMUS

CHIODI, H. Effect of castration on the thymus weight of adult rats (Acción de la castración de los adultos sobre el peso del timo de las ratas). *Rev. Soc. argent. de Biol.* 14: 435. 1938.

Male and female rats were castrated when 120 days old; they were killed 30, 45 and 60 days later and the weight of the thymus compared with that of normal controls. There was a marked hypertrophy with no qualitative changes in the microscopic aspect.—J. T. L.

LERICHE, R., AND A. JUNG. Thymectomy and parathyroidectomy for dystrophy of growth of the slender type with generalized calcinosis (Thymectomie et parathyroidectomie pour dystrophie de croissance du type gracilité avec calcinose généralisée). *Presse méd.* 46: 809. 1938.

A boy, normal to the 6th year, gradually developed changes consisting of a transparent white skin, extreme slenderness and fragility, thinness of the bones, tendency towards edema, muscle spasms and deposits of calcium, manifested as spots on the skin but also scattered through the body as shown by x-rays. The blood calcium varied between 10.5 and 15, the urinary calcium was extremely variable. Thymectomy and parathyroidectomy was performed at the age of 15. The histology of the removed material suggested thymus and thyroid [Parathyroid?] overactivity. Slow improvement and gradual subsidence of the calcium deposits followed the operation.—A. E. Meyer.

PENDE, N. Hyperthymism in infants and adolescents as related to abnormalities of development of sexuality and character (L'hyperthymisme des enfants et des adolescents dans ses rapports avec les anomalies du développement de la sexualité et du charactère). *Rev. méd.* 55: 175. 1938.

Hyperthymic infants are usually rather heavy at birth (4 to 5 kg.). They have a tendency toward allergic symptoms, eczema and intolerance to fats, eggs and butter. The sexual organs are small from the beginning. They are emotionally unstable, overactive and soon become obese. The muscles are poorly developed, the ligaments remain loose. Genu valgum is quite common. Boys show feminine traits. The sexuality does not develop at the usual age and the character development is typical as observed in hyposexual adolescents. The blood shows a relative lymphomonocytosis with granulopenia. The blood lipoids are increased. The enlarged thymus is diagnosed by means of a special percussion technic. The treatment consists in irradiation of the thymus and injections of posterior lobe pituitary extract, which is antagonistic to the thymus. The diagnosis is confirmed by the disappearance of the adiposity and the prompt onset of puberty after irradiation.—A. E. M.

SHAY, H., J., GERSHON-COHEN, S. S. FELS, D. R. MERANZE AND T. MERANZE. The thymus. Studies of some changes in the gonads and pituitary following its destruction by roentgen irradiation. *J. A. M. A.* 112: 290. 1939.

A summarizing article reporting that by x-ray irradiation in young rats (number not stated) considerable degrees of thymic destruction were induced. In males this was accompanied by arrest of spermatogenesis and sterility and by appearances in the pituitary similar to those following castration. Beginning at about 100 days restorative processes began and led to normality some 50 days later. In females no significant effects were observed.—R. G. H.

THYROID

BENJAMIN, B., AND P. R. MILLER. Hypothyroidism as a cause of disease of the hip. *Am. J. Dis. Child.* 55: 1189. 1938.

Disturbances of ossification involving the capital epiphysis of the femur may be present in one of two varieties in cretinism. 1. The structure of the epiphyseal plate is affected in such a manner as to give rise at times to slipping of the epiphysis. 2. Osteochondrosis of the head of the femur arises from an irregular, bony development of the epiphysis with persistence of numerous islands of uncalcified cartilage. In either case deformities ensue which lead to the appearance of coxa vara. Limitation of motion at the hip in children with hypothyroidism is presumptive evidence of hip disease. Four cases are reported and the literature reviewed.—M. B. G.

GESELL, A., C. S. AMATRUDA AND C. S. CULOTTA. Effect of thyroid therapy on the mental and physical growth of cretinous infants. *Am. J. Dis. Child.* 52: 1117. 1936.

The effects of thyroid substance on the cretinous infant are of three kinds: *a*) metabolic or dynamic, *b*) somatic and *c*) neurodevelopmental. Every cretin has a latent and distinctive optimal response which is probably favored by early treatment. The final influence on the cretin is therefore contingent not so much on the age of the child or his maturity status at the time of diagnosis as on the residual physiologic capacity and the latent growth potency of his neuro-endocrine system. Thyroid therapy cannot bring

about normality, if there has been a fundamental impairment of the system, hereditary or developmental. The initial response to treatment is part of a total symptomatology, and at the same time it is an index of the reserve capacity of the neuro-endocrine system.—M. B. G.

HILL, I. G. W. Some aspects of thyrotoxicosis and the results of treatment. *Edinburgh M. J.* 45: *Tr. Medico-Chirurgical Soc.* 213, 1938.

This paper, based on 238 cases from the medical wards covers carefully the general incidence, cardiovascular findings, and results of x-ray, radium, surgery, and medical measures used in the treatment. Surgery gave better final results than the other methods used.—J. C. D.

PEDERSEN, S., W. G. MADDOCK AND S. WINSLOW. Serum potassium in hyperthyroidism. *J. Lab. and Clin. Med.* 23: 1123. 1938.

With the method of Shohl and Bennett serum potassium data were obtained on patients with hyperthyroidism before and after operation. Two patients who developed thyroid crises were also included. In none of the cases studied was the serum potassium found to be abnormal at any time.—F. A.

READ, J. M. Studies in exophthalmic goiter. 1. Its incidence throughout the United States. *Arch. Int. Med.* 63: 71. 1939.

Statistical studies of the comparative incidences of endemic goiter and exophthalmic goiter in the United States are presented. These show that the latter occurs more uniformly throughout the country, even being present in regions where endemic goiter is unknown, which indicates that the 2 types of goiter are etiologically unrelated.—I. B.

SCHWARZ, C. On the relation between the thyroid and glycogen storage (Ueber die Beziehungen der Schilddrüse zum Zuckergehalt und zur Glykogenspeicherung). *Biochem. Ztschr.* 293: 295. 1937.

Thyroidectomized dogs have a diminished sugar tolerance which is corrected by thyroid feeding. The author believes that the diminished tolerance is due to the inability to store carbohydrates.—Emil Bozler.

SHORT, J. J. Extreme obesity followed by therapeutic reduction of two hundred and thirty-nine pounds. *J. A. M. A.* 111: 2196. 1938.

A woman weighing 395 lbs. but otherwise apparently normal was treated by reducing the diet to 600 calories a day then, as the 'specific dynamic action' of the foodstuffs waned, giving thyroid to correct the hypometabolism. The thyroid dosage was 3 grains daily. A weight of 156 lbs. was reached after 20 months without any evidence of ill-health. For cosmetic reasons considerable excess skin was removed surgically. In such cases, it is stated, thyroid is not well tolerated in the early stage of the treatment but is well tolerated after the reducing diet has got well into operation.—R. G. H.

WILKINS, L. The rate of growth, osseous development and mental development in cretins as a guide to thyroid treatment. *J. Pediat.* 12: 429. 1938.

The author uses a chart which compares the rates of growth (height age), osseous development (bone age) and mental development (mental age) of cretins under thyroid treatment. The developmental rates serve as more reliable guides to adequate thyroid treatment than measurement of B.M.R., blood cholesterol or the clinical picture. The bone development progresses more rapidly than growth and mentality; the latter may lag behind the normal rate. The observations in 6 'cretins' are reported.—M. B. G.